

UNIVERSITY OF ALBERTA

**Novel Chemistry of Amino Sugars:
Pathway to Glycosphingolipids and Glycopeptides**

by

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Abstract

Novel chemistry of 2-aminosugars has been investigated and the findings provided an efficient pathway to prepare biologically interesting glycosphingolipids and glycopeptides for immunochemical studies.

A new and direct approach leading to 2-acetamido-2-deoxy- β -D-glucopyranosides has been developed via a reactive furanose oxazoline. A variety of unprotected 2-acetamido-2-deoxy- β -D-glucopyranosides have been synthesized in just two steps from abundant D-GlcNAc. Glycosidation of alcohols is mild, highly stereoselective and compatible with a range of functional groups. The easy and large-scale preparation of the furanose oxazoline donor from D-GlcNAc allowed the synthesis of 2-acetamido-2-deoxy- β -D-glucopyranosides in a simple and practical manner.

The above approach was extended to the concise and large-scale synthesis of important and expensive 2-acetamido-2-deoxy- β -D-hexopyranosides. By suitable manipulation at the 3-position of the furanose oxazoline, three methyl 2-acetamido-2-deoxy- β -D-hexopyranosides (*galacto*, *allo*- and *gulo*-) and two methyl 2-acetamido-2,4,6-deoxy-2,4-diamino- β -D-hexopyranosides (*galacto*- and *gulo*-) were readily prepared via short sequences. Meanwhile, a biologically important muramic acid and a repeating β -D-GlcNAc (1 $\rightarrow$ 4)- β -D-MurNAc disaccharide unit of bacterial cell wall peptidoglycan were also synthesized from the furanose oxazoline. Both can be readily coupled with suitable peptides to afford muramylpeptides for use as immunoadjuvants and bacterial cell wall biosynthetic precursors.

Based on these new findings, a novel and flexible synthetic approach to C-18-phytosphingosine was developed via the key reactions of zinc-mediated reductive fragmentation and olefin cross metathesis (OCM). Using this approach, two kinds of unsaturated and saturated D-*xyl*o and natural D-*ribo*-phytosphingosines were conveniently synthesized from the corresponding 2-acetamido-2-deoxy- β -D-hexopyranosides. Another efficient approach to sphingolipids including truncated sphingosine, sphinganine and phytosphingosine was also explored by asymmetric aldol condensations using a chiral auxiliary.

Following these sphingolipid syntheses, the potent immunostimulant α -galactosylceramide (KRN7000) was successfully prepared by an OCM strategy in optimal and convergent routes. Improved glycosylation yields and excellent α -selectivity could be obtained with tetrabenzyl galactopyranosyl iodide donor and a truncated C-6-phytosphingosine acceptor. The truncated aglycon moiety was smoothly extended by OCM reactions in high yields. The OCM strategy was further applied to the synthesis of β -lactosylceramide, a common core structure of many glycosphingolipids (GSLs). The successful application of OCM in GSL synthesis provides us with a general and flexible route for rapid access to their analogues and derivatives.

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List of Abbreviations

Ac	acetyl
AcOH	acetic acid
Ala	Alanine
All	allyl
AllNAc	<i>N</i> -acetylallosamine, 2-acetamido-2-deoxy-allose
Ar	aromatic
Bn	benzyl
<i>t</i> -Boc	<i>t</i> -butoxycarbonyl
BODIPY	boron dipyrromethene difluoride
<i>t</i> -Bu	<i>t</i> -butyl
BSA	bovine serum albumin
Bz	benzoyl
CSA	camphor-10-sulfonic acid
CMP-Neu5Ac	cytidine-5'-monophospho- <i>N</i> -acetylneuraminic acid
DCM	dichloromethane
DIC	diisopropylcarbodiimide
DEAD	diethyl azodicarboxylate
DIBAL-H	diisobutylaluminium hydride
DIPEA	<i>N,N</i> -diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	<i>N,N</i> -dimethylformamide

DMM	dimethylmaleoyl
DMSO	dimethyl sulfoxide
DMTST	dimethyl(methylthio)sulfonium triflate
DTBS	di- <i>tert</i> -butylsilyl
EDCI	1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride
ELISA	enzyme-linked immunosorbent assay
ESI HRMS	electrospray ionization high resolution mass spectrometry
Et	ethyl
Fmoc	9-fluorenylmethoxycarbonyl
GalCer	galactosylceramide
GalNAc	<i>N</i> -acetylgalactosamine, 2-acetamido-2-deoxy-galactose
GCOSY	gradient coupling correlated spectroscopy
Glc	glucose
GlcNAc	<i>N</i> -acetylglucosamine, 2-acetamido-2-deoxy-glucose
Glu	glutamine
GSL	glycosphingolipid
HBTU	O-benzotriazole- <i>N,N',N'</i> -tetramethyl-uronium-hexafluorophosphate
HOBt	1-hydroxybenzotriazole
HMQC	heteronuclear multiple quantum coherence
HPLC	high pressure liquid chromatography
KLH	keyhole limpet hemocyanin
Lys	lysine
MALDI-TOF	matrix assistant laser desorption ionization time of flight

ManNAc	<i>N</i> -acetylmannosamine, 2-acetamido-2-deoxy-mannose
Me	methyl
MDP	muramyl dipeptide
MOM	methoxymethyl
MS	molecular sieves
Ms	mesyl
MurNAc	<i>N</i> -acetylmuramic acid
NAG	<i>N</i> -acetylglucosamine
NAM	<i>N</i> -acetylmuramic acid
NIS	<i>N</i> -iodosuccinamide
NMR	nuclear magnetic resonance
NMP	<i>N</i> -methyl-2-pyrrolidone
NKT	natural killer T
OCM	olefin cross metathesis
<i>i</i> -Pr	<i>iso</i> -propyl
R _f	retention factor
Ph	phenyl
Phth	phthalimido
Piv	pivaloyl
ppm	parts per million
Pyr	pyridine
RT	room temperature
SAR	structure-activity relationship

S_N2	biomolecular nucleophilic substitution
SepPak	solid phase extraction cartridge
TBAI	tetrabutylammonia iodide
TCR	T cell receptor
THF	tetrahydrofuran
TIPS	triisopropylsilane
Tf	trifluoromethanesulphonate
Tf ₂ O	trifluoromethanesulfonic anhydride
TFA	trifluoroacetic
TMS	trimethylsilyl
TLC	thin layer chromatography
Troc	2,2,2-trichloroethoxycarbonyl
TsOH	toluenesulfonic acid
TT	tetanus toxoid
UDP	uridine 5'-diphospho
UV	ultraviolet

Chapter One

Introduction: Glycosphingolipids and Amino Sugars

1.1 Glycoconjugates

Carbohydrates are the most abundant class of organic molecules found in living organisms. They are complex structures that exist in a wide range of forms from simple saccharides (glucose, mannose, sucrose etc) to large, energy containing polymers as well as in various biologically important glycoconjugate forms. In addition to their roles as energy sources and structural framework for all living cells, they are actively involved in a broad variety of biological processes as diverse as cell identification, movement, protein folding and much more.^[1,2]

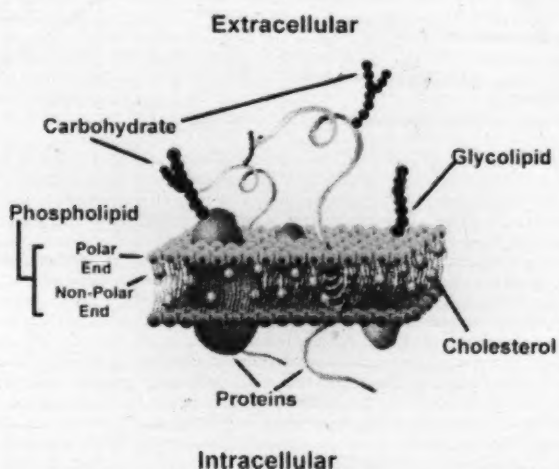


Figure 1.1 The schematic picture of glycoproteins and glycolipids on the cell surface

(http://www.harlem-school.com/10TH/sci_pdf/graphics/fluid_mosaic.gif)

Glycoconjugates are the general classification of carbohydrates covalently linked to other chemical species. Depending on the linked species and structures,

glycoconjugates are basically classified as glycolipids, glycopeptides, glycoproteins, and peptidoglycan or lipopolysaccharide of bacteria.^[2,3] They are densely distributed on the surface of virtually every cell in the mammalian body, and are also found on viruses, fungi, and bacteria. On the cell surface, carbohydrates or oligosaccharides are attached to proteins and lipids that are deeply embedded into the cell membrane as shown in Figure 1.1.

Surface carbohydrates on cells usually serve as initial points of attachment for other cells, infectious bacteria, viruses, toxins, hormones and many other molecules. Since cell surface carbohydrates constitute the outermost layer of the cell, glycoconjugates are widely involved in many interactions between the cell and its environment. It has been recognized that glycoconjugates on the cell surface play crucial roles in numerous biological recognition events and signal transduction such as bacteria and viral infection, receptors for toxins and hormones, fertilization, cell adhesion, cell differentiation and proliferation, tumor progression, aging, immune responses, brain-neural functions, and so on.^[2,4]

The involvement of carbohydrates in a variety of biological recognition events creates a number of opportunities for therapeutic intervention in cancer, tuberculosis, HIV, influenza and other important diseases.^[5-7] Several therapeutic strategies involving carbohydrates have been proposed: inhibition of carbohydrate processing enzymes that are up-regulated or present only in the disease state, interference with carbohydrate-protein or carbohydrate-carbohydrate interactions, immunization with carbohydrate antigens, or the exploitation of carbohydrate-protein binding specificity in drug delivery.^[7-9] Continued investigation of the biological recognition processes of

carbohydrates and glycoconjugates holds considerable promise for insights into rational design and development of novel carbohydrate-based drugs.

1.2 Glycosphingolipids

As a major class of glycoconjugates, glycolipids are essential membrane components and occur in most kingdoms of organisms, i.e., bacteria, plants, and animals including human beings. In general, glycolipids are amphiphilic biomolecules comprising a hydrophilic headgroup-carbohydrate linked by a glycosyl bond to apolar hydrophobic aliphatic lipid chains such as acylglycerol, ceramide, or prenol phosphate. Due to their amphiphilic character, glycolipids tend to aggregate into clusters in aqueous environments, as lipid bilayers or membranes. Together with glycoproteins and glycosaminoglycans they contribute to the glycocalyx that covers eukaryotic cells with a carbohydrate wall.^[10] Therefore, glycolipids are important factors of membrane stabilization and cell surface rigidity. They also play an important role in many biological processes, such as photosynthetic electron transport in plants, in cell-cell communication, in receptor modulation, and signal transduction.^[15] In general, glycolipids can be divided into two categories: one is the glycosphingolipids that are abundant in bacteria and plants, and the other is GSLs, also found in plants but mainly in animals. The latter is the focus of my research.

GSLs consist of a mono- or oligosaccharide unit and a ceramide, which is formed by attachment of a fatty acid by amide linkage to a long-chain amino alcohol sphingoid base. To date, numerous sphingoid bases have been isolated from natural resources and several major types of sphingoid bases are described in Fig.1.2. The most common

sphingoid base is *E*-sphingosine with a (2*S*, 3*R*)-*D*-*erythro* configuration, which is mainly found in animals and in some plants. *E*-Sphingosine is an unsaturated long chain amino alcohol (typically C-18) with a *trans* double bond joining C-4 and C-5 and its saturated counterpart is called as sphinganine or dihydrosphingosine. The corresponding lipid frequently found in plants is (2*S*, 3*R*, 4*R*)-*D*-*ribo*-phytosphingosine, in which the elements of water have been added across the double bond to give an additional hydroxyl at C-4. Besides the saturated phytosphingosine, there are also many examples of unsaturated phytosphingosine with an *E*-configured double bond at C-5 and C-6 found in plant GSLs. All of these sphingolipids have been proposed to play significant structural and regulatory roles in living organisms.^[11]

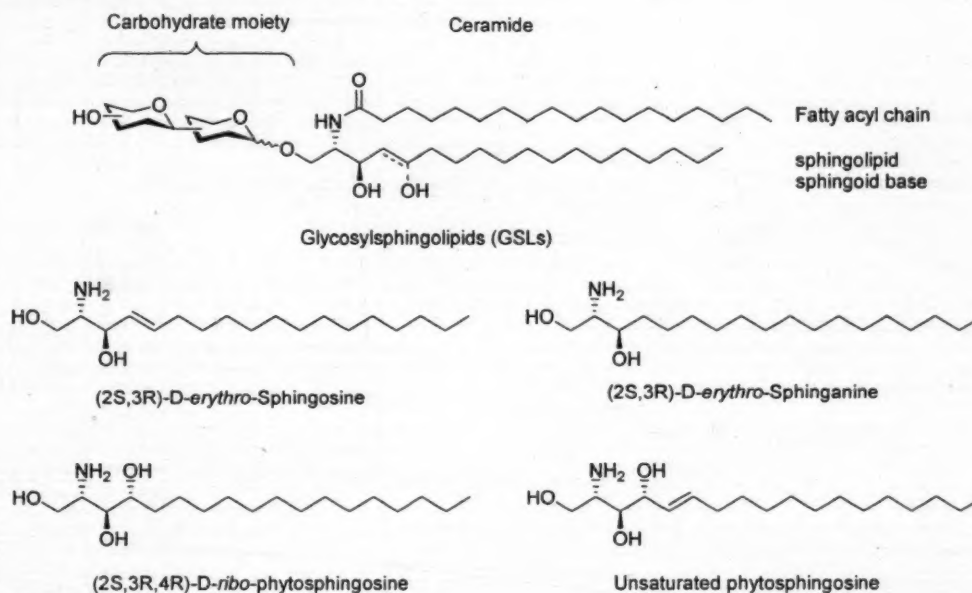


Figure 1.2 The structures of glycosphingolipids and major types of sphingolipids

Variations in ceramide structure and the type, number, and linkage of the sugar moiety give rise to a broad range of naturally occurring GSLs. More than 300 different

structures have now been characterized.^[12] In animal tissues GSLs are *N*-acylated (4*E*)-sphingosine derivatives and essential components of the plasma membrane of eukaryotic cell membranes. In plants, most glycosphingolipid analogues are phytosphingosine derivatives with linear saturated alkyl chains. They can be generally classified into the two groups on the basis of carbohydrate structures: neutral and acidic GSLs. Both comprise mono- or oligosaccharide sugar moieties. Negative charges in the latter group can be provided by sialic acids, uronic acid, sulfates, phosphates and phosphonates. Of the neutral GSLs, galactosylceramide is the most prominent member in the mammalian brain. Higher glycosylated GSLs are found in all tissues. They are grouped into distinct series, e.g., "ganglio", "globo", or "lacto" in order to distinguish them with regards to their structural and functional relationships.^[13]

The biosynthesis of ceramide and β -glucosylceramide is depicted in Figure 1.3.^[13,14] The condensation of L-serine with a fatty acyl coenzyme A, usually palmitoyl coenzyme A to 3-ketosphinganine, is catalyzed by the enzyme serine palmitoyl transferase (SPT). Then 3-ketosphinganine is reduced to *D-erythro*-sphinganine by the enzyme 3-ketosphinganine reductase and further acylated to dihydroceramide by the enzyme sphinganine *N*-acyltransferase. Dihydroceramide is subsequently desaturated to ceramide in the dihydroceramide desaturase reaction. Upon ceramide availability, a glucose residue is attached to the primary position of ceramide through the action of a glucosyltransferase utilizing UDP-Glc as the glycosyl donor. By treatment with galactosyltransferase and UDP-Gal donor, a galactose residue is further added to glucosylceramide affording a lactosylceramide, a common biosynthetic precursor for most GSLs series found in higher animals.

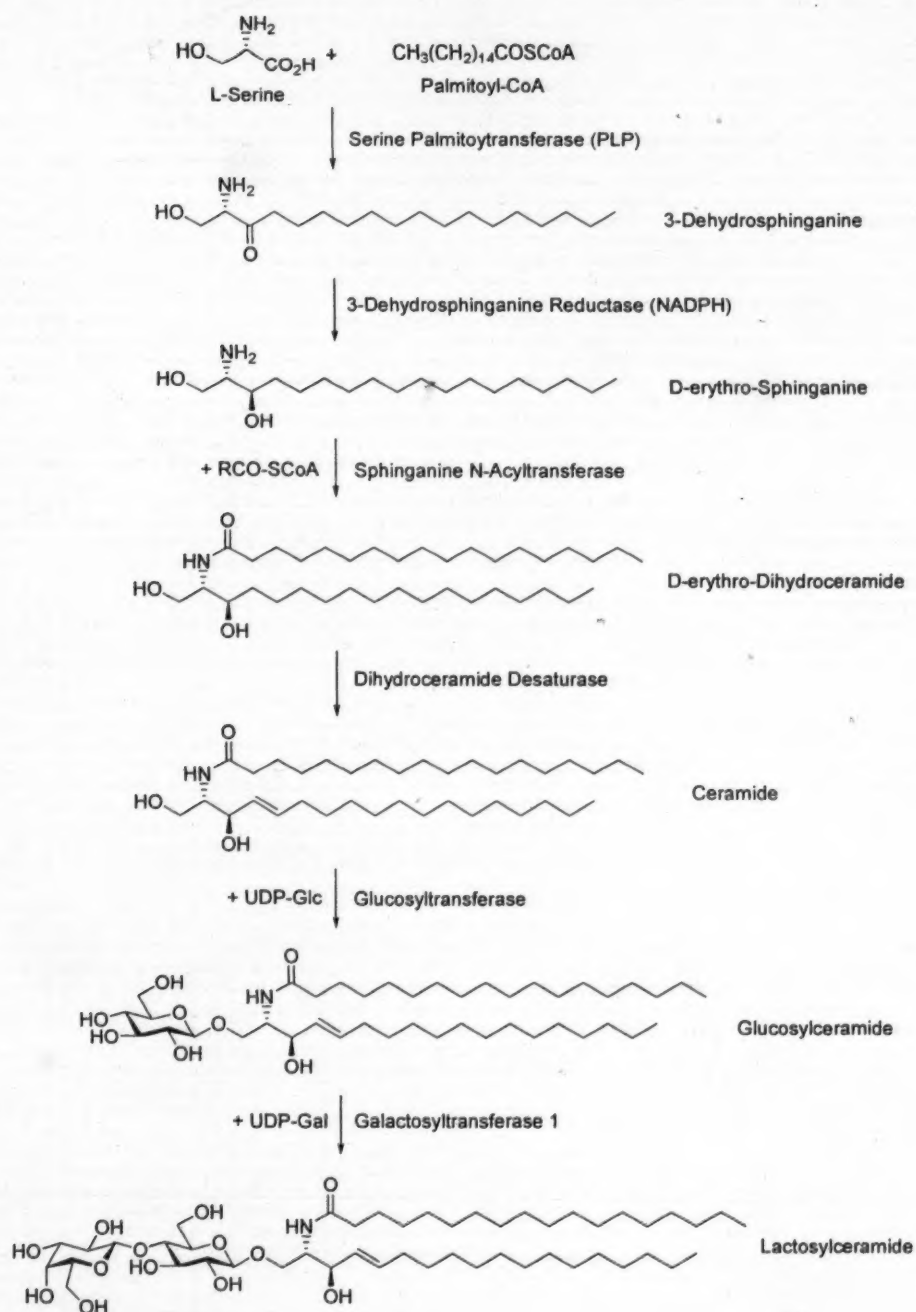


Figure 1.3. The biosynthesis of sphingolipids and β -lactosylceramides in nature

As mentioned above, GSLs are widely distributed in most mammalian membranes. Extensive studies have shown that GSLs play many important roles in a broad range of biological and physiological processes. Major functions of GSLs include cell-cell communications, regulation of cell growth and differentiation, mediation of signaling pathways, and stimulation of the mammalian immune system.^[15] Since our group is dedicated to developing novel carbohydrate-based vaccines, we have considerable interest in this class of compounds and their immunological functions. We are mainly concerned with two types of GSLs; one is α -galactosylceramide (also called KRN7000), and the other are the gangliosides, because both display significant immunological properties.

1.2.1 α -Galactosylceramide

Galactosylceramides are the simplest class of GSLs (cerebrosides) and important molecules existing in virtually all cells with almost exclusively the β -configuration. These β -galactosylceramides and their metabolites have been shown to possess important functions in promoting the regulation of nerve cells, regulating protein kinase C activities, and modulating the function of hormone receptors.^[16] Recently, β -galactosylceramide was identified as an essential component of the neural receptor for type 1 human immunodeficiency virus (HIV) surface glycoprotein gp120, mediating viral entry into epithelial cells.^[17] In addition, β -galactosylceramide has also been suggested as a possible ligand for the adhesion of *Helicobacter pylori* to cells in the gastric system.^[18]

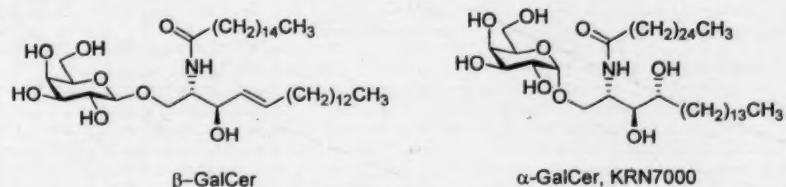


Figure 1.4. The chemical structures of α and β -galactoylceramides

In contrast to the common distribution of β -galactosylceramide in mammalian tissues, α -linked galactosylceramides are very rare in nature, and so far have not been detected in normal mammalian cells. α -Galactosylceramide was originally isolated from the marine sponge *Agelas mauritanus* in 1993 by the Pharmaceutical Division of the Kirin Brewery Company during a screen for reagents to prevent tumor metastases in mice.^[19] Compared with its β -linked counterpart, α -galactosylceramide has an unusual α -linkage between galactose and ceramide containing a C-18-phytosphingosine instead of *E*-sphingosine, which is commonly found in most β -glycoceramides.

In their initial studies some natural α -galactosylceramides were shown to have strong *in vivo* antitumor activities against several murine tumor cells.^[20] To investigate the relationship between their structures and antitumor activities, as well as to identify new candidates possessing antitumor activity; several analogues of natural α -galactosylceramide were synthesized. A synthetic analogue, designed as KRN7000 (Figure 1.4), was found to be a potent antitumor agent for clinical trials.^[16,21] Interestingly, further investigations revealed that KRN7000 could strongly stimulate natural killer T (NKT) cells via presentation by the protein CD1d,^[22] preventing autoimmune diseases such as Type 1 diabetes.^[23] The discovery of KRN7000 has

received extensive attention^[25] and in most studies KRN7000 is still referred to as α -GalCer, although this is an inaccurate abbreviation.

It was well known that antigen presentation to T cells plays a central role in adaptive and innate immunity.^[24,25] NKT cells are a unique subset of regulatory T cells that express both a conserved T cell receptor (TCR) and natural killer (NK) cell receptors. Unlike other T cells, NKT cells can recognize glycolipid antigens including α -GalCer when presented by the major histocompatibility complex (MHC) class I-like molecule CD1d. Upon recognition of the α -GalCer/CD1d complex through the T-cell receptor (TCR), NKT cells rapidly produce large amounts of T helper 1 (T_H1) and T helper 2 (T_H2) cytokines, such as interferon- γ (IFN- γ) and interleukin 4 (IL-4), respectively (Figure 1.5). The released cytokines play critical roles in inducing a series of cellular activation events leading to the activation of innate and adaptive immune cells.^[25-27] In the model of α -GalCer/CD1d complex, it is proposed that the lipid portion of α -GalCer interacts with the hydrophobic antigen-binding groove of CD1d. The carbohydrate portion of α -GalCer is accessible for interaction with the NKT-cell TCR. The crystal structures of CD1d binding with α -GalCer have revealed that the lipid chain of α -GalCer fits tightly into two hydrophobic pockets of the CD1d binding groove.^[28] Owing to their remarkable effects on the immune response, KRN7000 and other α -GalCer compounds have attracted considerable interest for development as vaccine adjuvants and for treatment of various pathologies, including cancer, infection, autoimmune and inflammatory conditions.^[29]

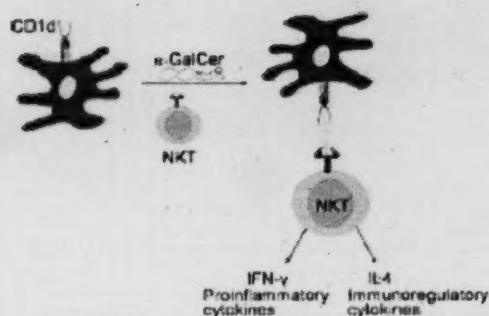


Figure 1.5. α -GalCer activates NKT cell via presentation by CD1d protein

(This picture is taken from Douglass Wu *et al. Proc. Natl. Acad. Sci. USA* **2005**, *102*, 1351-1356)

Since the discovery of α -GalCer, a number of analogues and derivatives have been synthesized (Figure 1.6) and evaluated to elucidate structure-activity relationships (SAR). At first, a series of α -galactosylceramides containing different monosaccharide, deoxy-monosaccharides and disaccharides as well as β -anomers were investigated.^[22] The preliminary studies have shown that both the galactose headgroup and the α -linkage are crucial for the ability of α -GalCer to act as a potent and efficient ligand for NKT cells. It was also found that the equatorial configuration of the 2-hydroxyl group of the sugar moiety is essential for recognition by the TCR of NKT cells, while some diversity can be accommodated at the 3- and 6-hydroxyl positions. For example, bacterial α -glycolipids containing a galactosyluronic acid or glucosyluronic acid could be also recognized by most mouse and human NKT cells.^[29,30] A 3-*O*-sulfo-analogue of α -GalCer was found to cause significant IL-2 secretion.^[31] Interestingly, the replacement of the anomeric oxygen of α -GalCer with other atoms can also alter the cytokine release profile. For instance, Franck's group synthesized a *C*-glycoside analogue of α -GalCer and it can strongly stimulate T_H1 response *in vivo* from NKT cells.^[32] More recently, an

S-glycoside analogue of α -GalCer has been synthesized in our group and its immunomodulatory studies are currently under study.^[33]

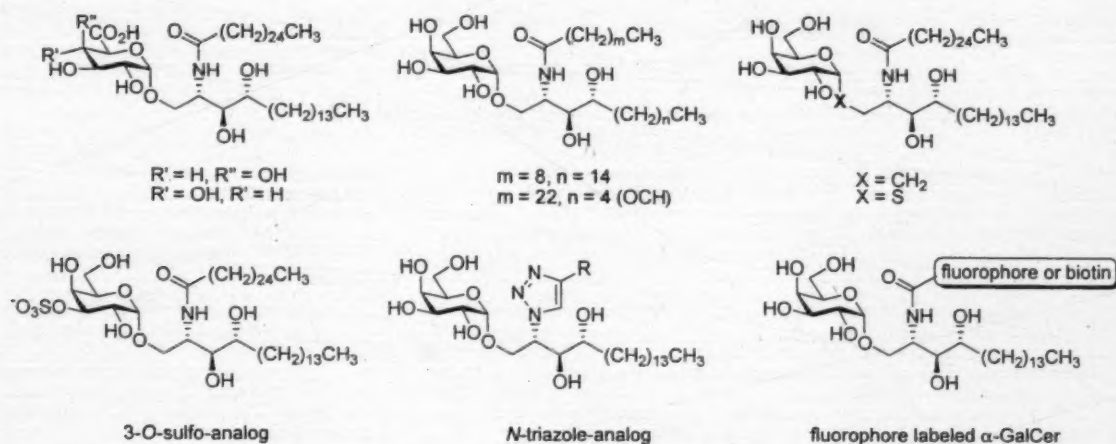


Figure 1.6. Representative synthetic analogues and derivatives of α -galactosylceramides

Besides modifications of the carbohydrate moiety, the structure of the ceramide moiety has also been studied. Modification of the fatty acid chain as well as the sphingoid base lead to changes in the cytokine release profile, presumably through alteration of glycolipid/CD1d complex stability. Porcelli *et al.* have reported that an α -GalCer analogue containing a diunsaturated C-20 fatty acid potently induced a T_H2 -biased cytokine response.^[34] Another α -GalCer analogue with a shorter fatty acid chain was found to have an increased T_H2 profile compared to that of KRN7000.^[35] Meanwhile, variation of the length of the phytosphingosine base has also been studied and a truncated analogue (known as OCH) was shown to be biased toward the T_H2 response, inducing a predominant production of IL-4.^[36] In addition, a 1,2,3-triazole containing α -GalCer analogue exhibits a stronger T_H2 cytokine response.^[37] However, the introduction of an

aromatic group to the fatty acid chain was found to enhance IFN- γ /IL-4 secretion and enables the tuning of T_H1/T_H2 cytokine profile.^[38] To elucidate the *in vivo* behavior of α -GalCer, a fluorescent BODIPY probe was introduced and found to be as active as KRN7000 for inducing apoptosis of liver NKT cells.^[39]

Although α -GalCer and related glycolipids hold considerable promise for the development of therapies for autoimmune disease, several obstacles remain before treatment with α -GalCer can progress from the bench to the bedside.^[25] To gain a better understanding of the immunological properties of human NKT cells and mechanisms by which treatment with α -GalCer modulate autoimmunity, various α -GalCer analogues and derivatives with therapeutic potential and combination therapies still need to be explored.

1.2.2 Gangliosides

Another important class of GSLs we are currently investigating as tumor associated antigens is the ganglio-series GSLs (Figure 1.7). The gangliosides are a family of over thirty acidic GSLs that contain at least one sialic acid residue, most commonly *N*-acetylneuramic acid, linked via a α -(2 $\rightarrow$ 3) or α -(2 $\rightarrow$ 6) glycosyl linkage to galactose or *N*-acetylgalactosamine units, or via an α (2 $\rightarrow$ 8) linkage to another sialic acid residue. Similar to many other GSLs, gangliosides usually have a common β -lactosylceramide core structure. Gangliosides are usually classified according to the nature of their carbohydrate component. The widely accepted Svennerholm nomenclature refers to the number of sialic acid residues (M = mono, D = di, T = tri, Q = tetra).^[40] Gangliosides were initially isolated and characterized from bovine brain and are normal components of the outer surface of virtually all mammalian cell membranes. They play a significant role

in a variety of cell functions such as cell growth and differentiation control, mediation of cell-cell interactions, regulation of cell signaling by acting as receptors for growth factors, and acting as specific receptors for various microbial toxins.^[2,15] Furthermore, these glycolipids have found wide applications in oligosaccharide microarrays, investigations of sialic acid binding proteins, immunotherapy, and in the study of cell membrane events.^[41]

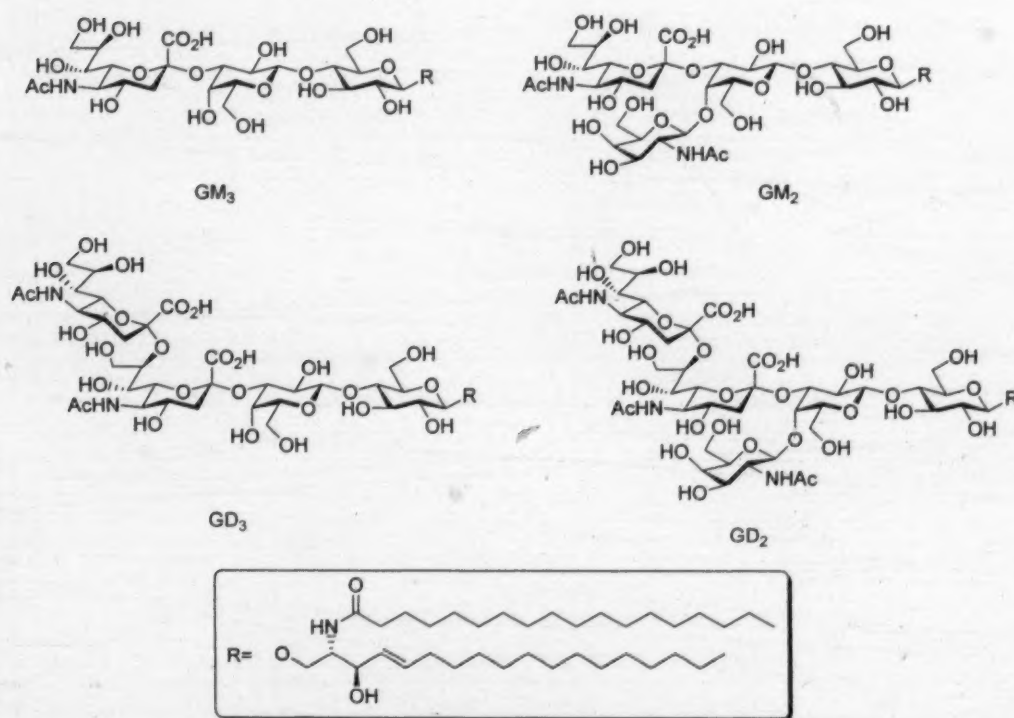


Figure 1.7. The structures of some tumor associated ganglioside antigens

Importantly, many gangliosides, in particular GM_3 , GM_2 , GD_3 and GD_2 , are significantly overexpressed in cancer tumors like melanoma and other tumors of neuroectodermal origin. Therefore the structures of tumor associated antigens are potential targets for active cancer immunotherapy.^[42] Gangliosides have been considered

as promising immunotherapeutic targets due to the accessibility of the carbohydrate to the immune system, the high concentration of antigen on malignant cells, and their widespread expression in tumors from various patients. Unfortunately, there are several disadvantages of ganglioside-based immunotherapy such as low levels of immune response, the T-cell independent nature of unmodified immunogen, immune system tolerance and potential autoimmune responses.^[42a] all of these problems need to be circumvented before gangliosides are developed as an effective and practical immunotherapy for cancer.

A variety of strategies have been developed in the elaboration of ganglioside-based vaccines to address the above issues associated with gangliosides as immunogens. Several successful approaches include conjugation of gangliosides to appropriate immunogenic carrier proteins, co-administration of highly immunostimulatory adjuvant preparation, and subtle modification of the ganglioside antigen.^[43] The strategy of conjugating an oligosaccharide epitope to an immunogenic carrier protein has been widely employed to enhance the immunogenicity of otherwise T-cell independent carbohydrate antigens. Several naturally occurring gangliosides or semi-synthetic ganglioside-based vaccines have been developed by conjugation to the carrier protein keyhole limpet hemocyanin (KLH) and investigated in the clinical trails.^[44] Although an increased immune response has been elicited in some ganglioside-KLH conjugates, they display only limited success in clinical trials. Therefore, new strategies for the design of ganglioside-based vaccines are still required in order to further improve their immunogenicity.

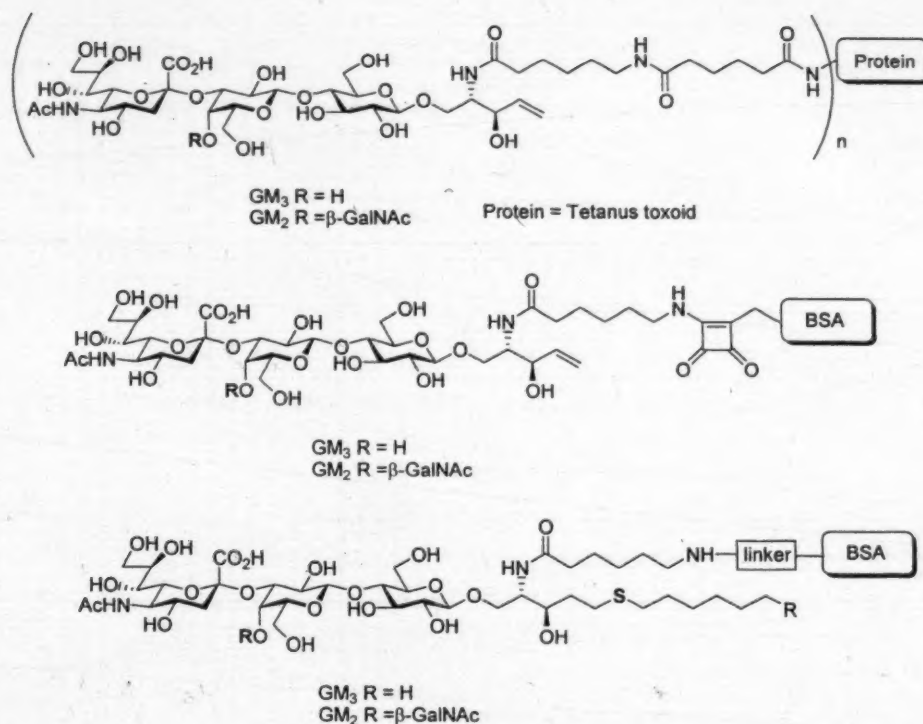


Figure 1.8. Several novel ganglioside-based antigens developed in our group^[41,45,46]

The synthesis of gangliosides is particularly challenging due to their amphiphilic nature and the difficulties associated with stereoselective coupling of *N*-acetyl neuramic acid. In order to circumvent these synthetic problems, a versatile chemoenzymatic approach to ganglioside analogues in a form suitable for conjugation has recently been developed in our group.^[40,45] In this approach, a truncated version of sphingosine was introduced to facilitate the coupling of neogangliosides to a carrier protein while maintaining the stereochemical integrity and structural features of naturally occurring ceramide. Furthermore, the introduction of truncated ceramide aglycon was intended to avoid extensive hydrophobicity that can hamper synthesis and purification of the glycolipids. Several analogues of GM_3 and GM_2 gangliosides bearing a truncated ceramide aglycon were chemoenzymatically synthesized and conjugated to tetanus toxoid

(TT) and bovine serum albumin (BSA) via linkers indicated in Figure 1.8 for immunization of mice.^[40,45,46] It was shown that mice immunized with GM₃-TT and GM₂-TT conjugates gave a high IgG hapten-specific response, and antibodies generated by GM₃-TT and GM₂-TT conjugates exhibited specificity for the carbohydrate epitope and the stereogenic centers of the ceramides. These truncated ceramide-containing ganglioside analogues provide a valuable tool for the design and development of cancer vaccines, as well as the study and modulation of ganglioside functions at membrane interfaces.

The carbohydrate moiety of gangliosides also needs to be subtly modified to enhance the immunogenicity of these tumor-associated antigens. It was known that some tumor-associated carbohydrate antigens contain glycosidic linkages that are potentially susceptible to endogenous glycosyl hydrolases, for example, ganglioside-specific plasma-membrane sialidase.^[47] The loss of terminal sialyl residues would destroy crucial recognition elements and thereby might result in poor immunogenicity. Recently, to improve the hydrolytic stability, we have modified the common terminal glycosidic linkage of GM₃ and GM₂ by replacement of the interresidue oxygen atom by sulfur using chemical or enzymatic methods (Figure 1.9).^[45,46] Two *S*-linked ganglioside GM₃ and GM₂ analogues containing a truncated ceramide aglycon have been prepared and attached to carrier protein TT or BSA via a common conjugation approach. Immunization experiments of these *S*-linked conjugates indicated that *S*-linked immunogens clearly generate carbohydrate-specific response that approach or exceed the response to the native oligosaccharide. We anticipate that antigens bearing hydrolysis-resistant *S*-linkages will have potential application in active cancer immunotherapy.

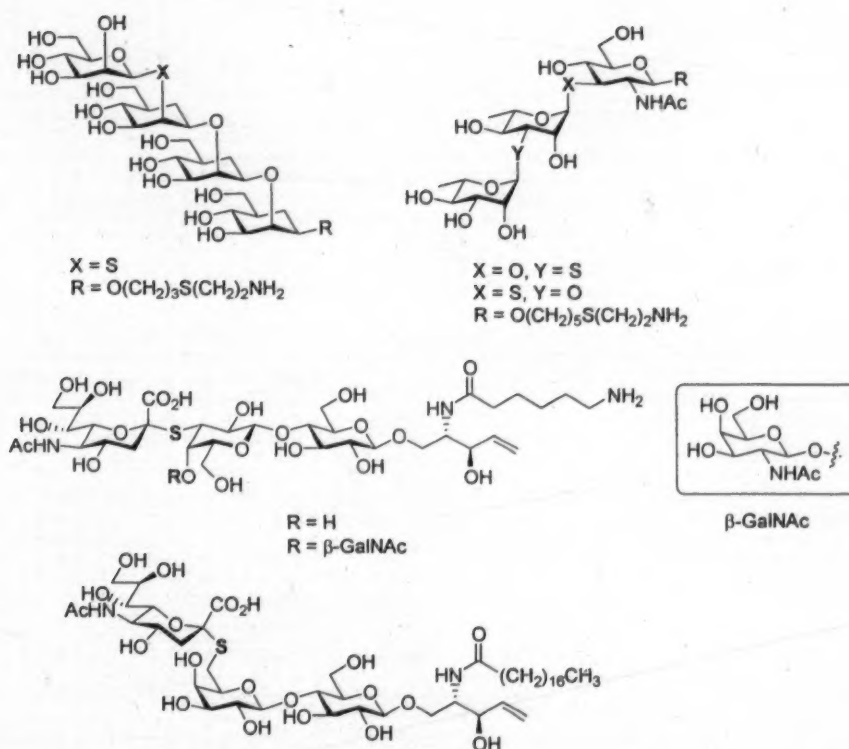


Figure 1.9. Thio-linked synthetic carbohydrate antigens developed in our group

1.3 Amino sugars in nature

It is well known that 2-amino-2-deoxyhexoses are among the most important modified sugars found in nature. In most cases, 2-amino-2-deoxyhexoses are found as the acetamido derivatives.^[48] Both of 2-amino and 2-acetamido sugars are crucial to the well being of many organisms, including humans, because they play pivotal structural and functional roles. The presence of a free amine in monosaccharide residues allows for protonation and the generation of a positively charged ammonium ion; the acetamido group, although far less basic than an amine, still imparts a good deal of polarity to the molecule.

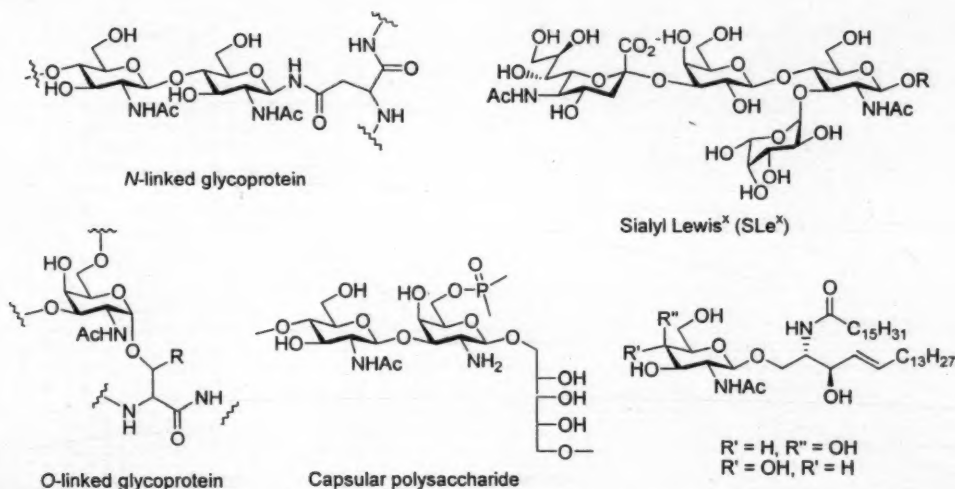


Figure 1.10 Selective examples of oligosaccharides and glycoconjugates containing D-GlcNAc and D-GalNAc

The most common and abundant naturally-occurring 2-amino-2-deoxyhexose is *N*-acetyl-D-glucosamine (D-GlcNAc), which is widely distributed in living organisms where it is a ubiquitous constituent of biologically-important oligosaccharides and glycoconjugates, including glycoproteins, glycopeptides, glycolipids, peptidoglycan, and glycosaminoglycans. Another common naturally occurring structure is *N*-acetyl-D-galactosamine (D-GalNAc). Both D-GlcNAc and D-GalNAc are frequently found in glycoconjugates, especially cell surface glycoproteins. The most relevant glycosyl-peptide linkages occurring in glycoproteins are the *N*- and *O*-linked structures shown in Figure 1.10. For example, the *N*-linked glycan of glycoprotein contains a disaccharide GlcNAc-GlcNAc as the core structure that is attached to an asparagine residue of the protein, and D-GalNAc is the first monosaccharide anchored to the serine or threonine residue of a protein carrier in the *O*-linked glycan of glycoprotein. D-GlcNAc and D-

GalNAc are also encountered in human milk, in blood group substances such as the ABO and sialyl Lewis X, in bacterial lipopolysaccharide antigens where they constitute part of epitopes, and in plants as well as a number of natural products.^[48,49]

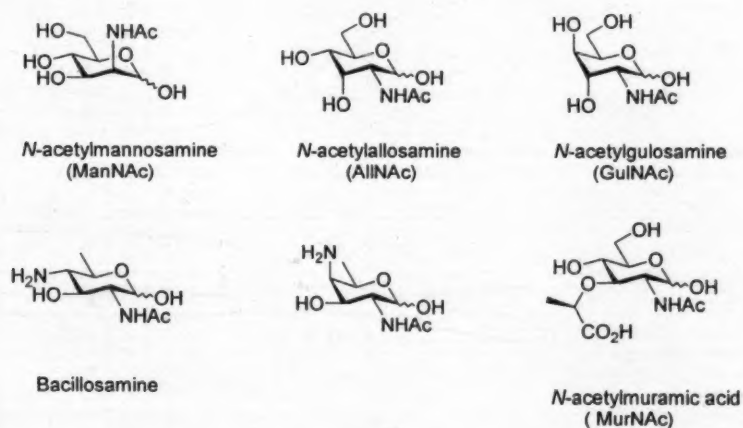


Figure 1.11. Several important naturally occurring 2-acetamido-2-deoxyhexoses and derivatives

Besides D-GlcNAc and D-GalNAc, there are several other 2-amino-2-deoxyhexoses and their derivatives found in nature, such as *N*-acetyl-D-mannosamine (ManNAc), *N*-acetyl-D-allosamine (AlNAc), *N*-acetyl-D-gulosamine (GulNAc), and 2,4-diamino-2,4,6-trideoxyhexoses as well as *N*-acetylmuramic acid (Figure 1.11).^[50] D-ManNAc is the biosynthetic precursor of common terminal sugars found on surface glycoproteins in many mammalian, bacterial and living cells. Both D-AlNAc and D-GulNAc exist in some important inhibitors and antibiotics. 2,4-Diamino-2,4,6-trideoxyhexoses are frequently found as important constituents in many bacterial polysaccharides. *N*-Acetylmuramic acid, a derivative of *N*-acetylgalactosamine, is a major component of the bacteria cell wall peptidoglycan. These 2-amino-2-deoxyhexoses exhibit a wide range of important biological and physiological properties. While *N*-

acetyl-D-glucosamine is plentiful and cheap, such is not the case for most other 2-amino-2-deoxyhexoses including even D-GalNAc.^[51]

1.4 Scope of projects

In the following chapters, novel chemistry of 2-amino-2-deoxyhexoses has been developed and applied to the flexible and efficient synthesis of sphingolipids (phytosphingosines and sphingosine), GSLs (α -galactosylceramide and β -lactosylceramide) and glycopeptides (muramylpeptides) for use as immunomodulatory agents and adjuvants.

At first, a facile two-step approach to 2-acetamido-deoxy- β -D-glucopyranosides from abundant D-GlcNAc was developed via a reactive furanose oxazoline. A variety of important and useful 2-acetamido-deoxy- β -D-glucopyranosides have been prepared through this approach. Secondly, this approach was further extended to the large scale and expedient synthesis of some rare but important 2-amino-2-deoxyhexoses and derivatives including 2,4-diamino-2,4,6-trideoxyhexoses and *N*-acetylmuramic acid, a repeating disaccharide unit of bacterial cell wall as well as a muramyl dipeptide.

Then, starting from 2-amino-2-deoxy- β -D-hexopyranosides obtained from above, several C-18-phytosphingosine diastereomers including the natural *D-ribo*-configuration were rapidly synthesized through an olefin cross metathesis (OCM) chain elongation strategy involving zinc-mediated reductive fragmentation as a key reaction. In principle, this method can be utilized to prepare a broad variety of phytosphingosine analogues, derivatives as well as diastereomers. In addition, another effective method of asymmetric aldol condensation was also exploited in this research to prepare several important

sphingolipids including truncated sphingosine, sphinganine as well as two phytosphingosines.

Next, a convergent synthesis of α -galactosylceramide (KRN7000) was explored starting from the corresponding 2-amino-2-deoxy- β -D-hexopyranosides with subsequent use of OCM strategy. In this context, a galactosyl iodide was employed as the donor to realize the desired glycosylations in high yields and excellent α -selectivity. Furthermore, the OCM strategy was also successfully applied to the efficient elaboration of β -lactosylceramide, a common core structure found on gangliosides and many other GSLs.

Chapter Two

Facile Synthesis of 2-Acetamido-2-deoxy- β -D-glucopyranosides via a Furanose Oxazoline Derivative

2.1 Introduction

2-Acetamido-2-deoxy-D-glucose (*N*-acetyl-D-glucosamine, D-GlcNAc) is one of the most frequently encountered sugar units in biologically-important complex oligosaccharides and glycoconjugates, including glycoproteins and glycolipids, glycosaminoglycans and peptidoglycans. It is also encountered in human milk, in blood group substances, in bacterial lipopolysaccharide antigens where it constitutes part of the epitopes, and in plants. D-GlcNAc was initially considered as a major structural constituent in cell membranes, but is now found to be associated with a wide range of biological processes and plays a key role in molecular and cell recognition and signaling. Owing to its wide occurrence in nature and biological significance, the synthesis of 2-acetamido-2-deoxy-D-hexosides has been a major task in carbohydrate chemistry for decades.^[51]

In nature, D-GlcNAc exists mainly in the pyranose form, and in the majority of cases it is found as either *N*- or *O*-linked β -pyranosides.^[52] However, the chemical synthesis of *O*-linked β -glucopyranosides of D-GlcNAc is much more difficult than its D-glucose counterpart. The conventional synthesis of D-GlcNAc β -glucopyranosides from D-GlcNAc utilizes the acetamido protecting group.^[49,53,56] This direct synthesis typically involves glycosylations of simple alcohols by reaction with triacetylated *N*-acetylglucopyranosyl chloride **2** under Koenigs-Knorr^[55] conditions (Figure 2.1). The

chloride donor **2** is prepared by reacting *N*-acetyl-D-glucosamine with neat acetyl chloride.^[54] However, there are several drawbacks to this approach. First, the reaction of D-GlcNAc with acetyl chloride is not clean; usually some of the pentaacetate **3** is formed, together with the desired chloride.^[56] Recrystallization does not remove this side-product. Consequently, the impurity **3** has to be removed by chromatography, or alternatively the crude chloride **2** is used for glycosylation, and the impurity **3** has to be removed in the next step. However, flash chromatography of polar acetamido intermediates is troublesome because they have poor solubility in common organic solvents and streak badly on TLC. Secondly, the glycosylation often employs poisonous heavy metal salts such as mercury cyanide or mercury chloride as a promoter. Thirdly, the glycosylations can also give the pyranose oxazoline **4** as a side product.^[49,58]

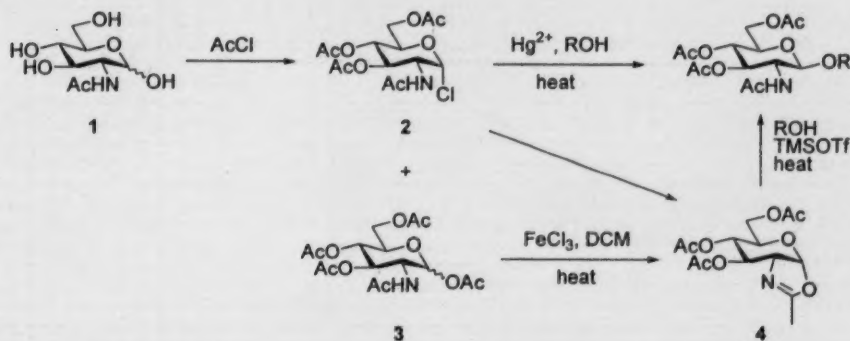


Figure 2.1. The conventional synthesis of 2-acetamido-2-deoxy-β-D-glucopyranosides from D-GlcNAc

Another direct approach using the acetamido group involves the use of pyranose oxazoline **4** as the donor.^[58] The oxazoline **4** can be prepared from D-GlcNAc pentaacetate **3**^[59] or the corresponding chloride donor **2**.^[49,58] In fact, oxazoline **4** is generally considered as the intermediate that leads to β-glycosides when the glycosyl

chloride **2** reacts with an alcohol.^[57] Unfortunately, this pyranose oxazoline **4** has proven to be a poor glycosyl donor due to its high stability and low reactivity.^[49,58] Usually, harsh reaction conditions using strong Lewis acids (such as TMSOTf) or strong Brønsted acids (such as *p*-toluenesulfonic acid) as well as elevated temperatures are required for glycosylations. Although some improvements were achieved using 1, 2-dichloroethane as solvent^[60] and use of FeCl₃,^[61] CuCl₂^[62] or Yb(OTf)₃^[63] as catalysts, elevated temperature is still needed and the glycosylations are restricted to particular acceptors. Recently, Boysen *et al.* designed a bicyclic 2,3-*N,O*-carbonyl thioglycoside as a versatile D-GlcNAc donor^[64] and Mohan *et al.* employed microwave as an efficient way of heating to convert a pyranose oxazoline derivative to some *N*-acetylactosamine compounds.^[65] Indeed, the preparation of β-glucopyranosides of D-GlcNAc with simple aglycones by traditional direct approaches that start from D-GlcNAc is not a straightforward task.

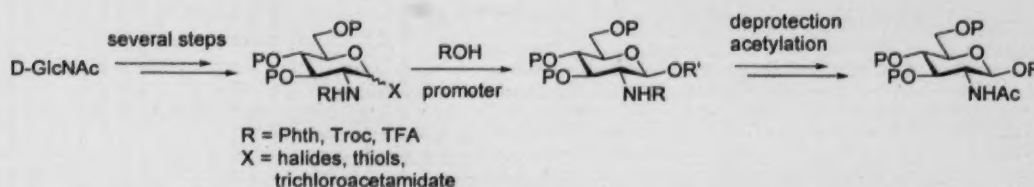


Figure 2.2. The commonly used indirect strategy for the synthesis of 2-acetamido-2-deoxy-β-D-glucopyranosides

Because the acetamido group is a poor participating group as mentioned above, indirect methods are commonly used in current methodologies for the synthesis of *O*-linked D-GlcNAc β-glycosides, especially when the acceptor is a complex sugar.^[49] These indirect methods most often employ a temporary protecting group for the 2-amino functionality such as phthalimido-,^[66] tetrachlorophthalimido-,^[67] Troc-amido,^[68]

chloroacetamido-^[69] trichloroacetamido-^[70] and Fmoc groups.^[71] However, as described in Figure 2.2, a multi-step procedure of protecting group manipulation on 2-amino-2-deoxy-hexosyl donor is required. Furthermore, after glycosylations, temporary protecting groups need to be removed and reacylated to provide the natural acetamido group. Although better yields can be obtained from the glycosylation step owing to the introduction of good participating groups, these indirect approaches are obviously not straightforward when considering their lengthy manipulation sequences.

In this context, we developed a simple and general two-step method for the preparation of 2-acetamido-2-deoxy- β -D-glucopyranosides from D-GlcNAc via a reactive furanose oxazoline derivative.^[72]

2.1 The discovery of glycosylation using furanose oxazoline 5 as the donor

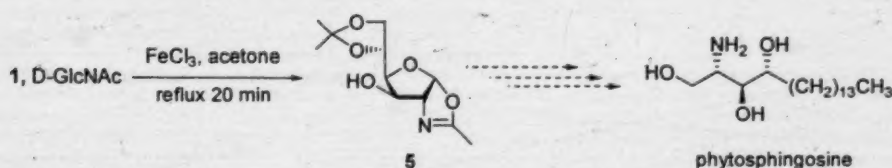
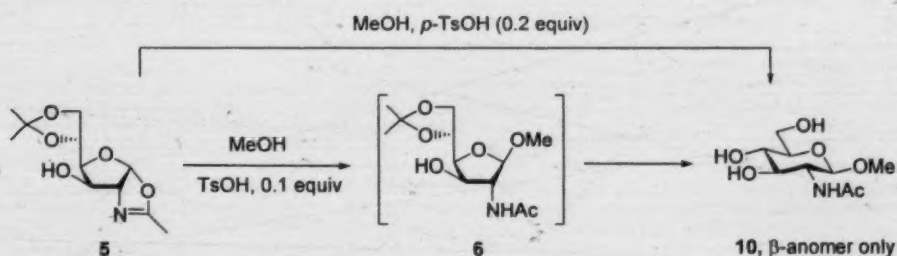


Figure 2.3. The proposed synthetic plan of phytosphingosine from D-GlcNAc

During our initial synthesis of bioactive phytosphingosines and glycosylceramide derivatives from inexpensive D-GlcNAc, we envisioned the use of a furanose oxazoline 5 as a key intermediate (Figure 2.3). Compound 5 can be conveniently prepared from *N*-acetyl-D-glucosamine with dry acetone using anhydrous FeCl₃ as catalyst;^[73] the reaction can be carried on a large scale in good yield (30 g, 77%). The crude furanose oxazoline 5 is pure enough for use in the next step without further

chromatographic purification. The literature reported that the furanose oxazoline **5** could be converted to the methyl β -furanoside **6** when reacted with dry methanol (as solvent) for 3 hours at pH 3~4 in the presence of *p*-TsOH as catalyst.^[74] However, our experience indicated that under the reported conditions, the furanoside **6** forms initially, then is slowly converted to a new spot on TLC identified as methyl 2-acetamido-2-deoxy- β -D-glucopyranoside **10** (Scheme 2.1). The β -anomeric configuration of **10** was unambiguously established by the large $^3J_{1,2}$ coupling constant (8.5 Hz). The rate for conversion of **6** into **10** accelerates if the amount of *p*-TsOH is increased. For example, if 0.5 equiv of *p*-TsOH is used, oxazoline **5** can be converted to compound **10** within 1 hour. The 5,6-*O*-isopropylidene group is apparently transacetalized *in situ* to methanol. In order to stop the transformation at the stage of furanoside **6**, the acidity of the reaction has to be carefully controlled.



Scheme 2.1. Synthesis of methyl 2-acetamido-2-deoxy- β -D-glucopyranoside **10** from furanose oxazoline **5**

The direct transformation of the furanose oxazoline **5** to the β -pyranoside **10** proceeds equally well on a small or large scale. When performed on a 20-gram scale, compound **10** was obtained in quantitative yield by simply evaporating the reaction mixture after neutralization with Et_3N , followed by multiple washing of the residue with

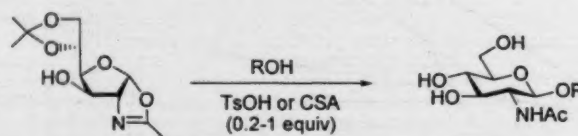
dichloromethane in order to remove *p*-TsOH·NEt₃ salts. Furthermore, oxazoline **5** could be prepared from D-GlcNAc on a large scale without chromatography. This now provides a valuable two-step procedure to prepare compound **10**, which is otherwise troublesome to make using conventional approaches. A literature search revealed that, interestingly, there are no other routes to directly convert a furanose oxazoline **5** to 2-acetamido-2-deoxy-β-D-glucopyranosides, although Gigg's group has reported a similar transformation using a furanose phenyloxazoline derivative to prepare two β-glycosides of GlcNBz.^[75]

2.3 Studies of glycosylation scope

Compared to the conventional methods that require at least three transformations as well as multi-chromatographic steps, this new route requires only two steps without chromatography to prepare unprotected 2-acetamido-2-deoxy-β-D-glucopyranosides from *N*-acetyl-D-glucosamine. The concise method, its convenience, and high stereoselectivity prompted us to explore the scope of this reaction. We first investigated the reaction of furanose oxazoline **5** with commercially available simple alcohols (Table 2.1, entry 1-8). In general, oxazoline **5** reacted smoothly with all the primary alcohols to afford exclusively β-glucopyranosides (entry 1-5, Table 2.1). No α-anomer was observed from these primary alcohols. With smaller and more reactive alcohols such as allyl, benzyl, ethylene glycol, we used 0.5 equiv of anhydrous *p*-TsOH or camphor-10-sulfonic acid (CSA) as catalyst, while for larger and less reactive alcohols, we increased the amount of *p*-TsOH or CSA to 1 equivalent in order to accelerate the reactions. Besides primary alcohols, secondary and tertiary alcohols were also examined with oxazoline **5** (entry 6-8,

Table 2.1). For instance, when cyclohexanol was used (entry 6), the corresponding cyclohexyl methyl 2-acetamido-deoxy- β -D-glucopyranoside was obtained in 73% yield as the only product. However, small amounts of α -isomers were observed in the cases of isopropanol and *t*-butyl alcohol. The reaction with *t*-butyl alcohol was sluggish. Remarkably, despite steric hindrance, the yield of the *t*-butyl glucoside was moderate.

Table 2.1. Glycosidation of furanose oxazoline **5** with simple alcohols^c



Entry	Glycosyl acceptors	Solvent ratio ^a	Yields ^b	α/β ratio
1	Allyl alcohol	1:0	82% ^c	0:1
2	Benzyl alcohol	1:0	73% ^c	0:1
3	1-Octanol	1:0	69%	0:1
4		1:0	78% ^d	0:1
5		1:0	77% ^d	0:1
6		1:0	73%	0:1
7	Isopropyl alcohol	1:0	77% ^c	1:6
8	<i>t</i> -Butyl alcohol	1:0	51%	1:4

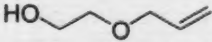
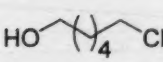
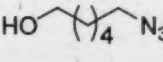
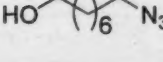
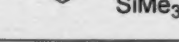
^a ROH:CH₂Cl₂; ^b yields are reported after chromatography; ^c Glycosides could also be obtained without chromatography; yields are ~ 10% lower; ^d No dimmers was detected;

^e All products and data are listed in the experimental section without numbers.

It was also found that the glycosylations were compatible with a variety of

functional groups (Table 2.2). For example, alkenes, halides, trimethylsilylethyl, and azido groups are all tolerated. Good yields together with complete β -stereoselectivity were obtained from these functionalized alcohols. The resulting β -glycosides bearing functional groups like alkene, azido- and halides are actually very useful intermediates in the construction of complex D-GlcNAc containing neoglycoconjugates.^[72b,77]

Table 2.2. Glycosidation of furanose oxazoline **5** with functionalized alcohols^d

Entry	Glycosyl acceptors	Solvent ratio ^a	Yields ^b	α/β ratio
1		1:0	76% ^c	0:1
2		1:0	74%	0:1
3		1:0	65%	0:1
4		1:0	63%	0:1
5		1:0	72%	0:1
6		1:2	70%	0:1
7		1:2	67%	0:1
8		1:2	61%	0:1

^a ROH:CH₂Cl₂; ^b yields are reported after chromatography; ^c Glycosides could also be obtained without chromatography; yields are ~ 10% lower; ^d All products and data are listed in the experimental section without numbers.

The glycosidations of oxazoline **5** were normally carried out in neat alcohol solution as shown in Table 2.1. and 2.2. However, for several more expensive alcohols (entry 6-8, Table 2.2), the reaction was performed using dichloromethane as a co-solvent

and no decrease in stereoselectivity was observed. For those volatile alcohols of smaller size (entries 1, 2, 7, 8 in Table 2.1 and entry 1 in Table 2.2), an excess of alcohol was removed by evaporation after the glycosylations. The desired pure β -glycoside products could be obtained after multiple washing with dichloromethane and no chromatography is required. However, as the size of the aglycones increases, the corresponding alcohols tend to be less volatile, and the glycosides have better solubility in dichloromethane, so these β -glycosides were isolated by chromatography.

2.4 Preliminary studies on glycosylation mechanism

The mechanism of glycosylation using furanose oxazoline donor **5** was then investigated. The reaction of a furanose derivative to form a pyranoside requires the anomeric acetal of the furanoside to be opened and formation of a new acetal linkage with the 5-hydroxyl group;^[76] logically, this should require the formation of an open-chain D-GlcNAc intermediate. The ring re-closure of the linear intermediate should afford, in principle, a mixture of anomeric glycosides; in the case of pyranosides. The α -anomers are usually the predominant products due to the anomeric effect as seen for example in Fisher glycosidation of D-GlcNAc.^[78] However, our results clearly showed that there was a preference for β -glucopyranoside formation when the furanose oxazoline **5** was reacted with alcohols. During the reaction of **5** with methanol, two intermediates were observed by TLC (5:1 CH₂Cl₂-MeOH): The top spot, methyl β -D-furanoside **6** (R_f = 0.72), appeared first. Before the complete disappearance of **6**, another lower spot appeared (R_f = 0.30), which was concomitantly converted to the β -pyranoside **10** (R_f = 0.16). We were able to halt the reaction by treating the reaction mixture with Dowex-2

(OH⁻) resin to remove the acid catalyst and the lower spot was isolated and identified as the triol **7**. Consequently it was concluded that the furanose oxazoline ring was first opened by the attack of methanol under acidic condition to yield **6** and subsequently the acetonide was transacetalized to methanol to afford **7**, which then rearranged to **10** through a faster process (Figure 2.4). Unfortunately, no intermediates could be observed during the transformation of **7** to **10**. The transformation of methyl furanosides **6** as well as **7** to **10** was first reported in the 70's by Jacquinet *et al.*^[79] They discovered that these two methyl furanosides could rearrange quantitatively to pyranoside **10** by treatment with 60% aqueous acetic acid solution at 100 °C for 10 min. In spite of its efficiency, this method has not found widespread use.

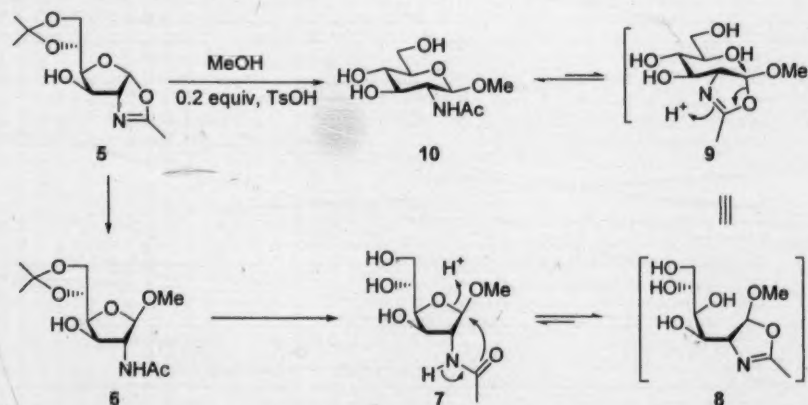


Figure 2.4. The postulated glycosylation mechanism of furanose oxazoline **5**

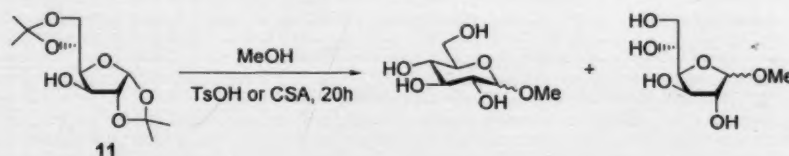
In order to explain the β -selectivity of glucopyranoside formation we speculate that there is an assisted ring opening of the furanoside **7** from the acetamido group (Figure 2.4). Thus, under *p*-TsOH catalysis, the ring oxygen of **7** is first protonated, and undergoes a 2-acetamido-assisted ring opening; The carbonyl oxygen attacks the anomeric center from the opposite side of the ring and inverts the anomeric configuration

to form an open-chain oxazoline intermediate **8**, which quickly undergoes another ring closure with 5-OH to regenerate the acetamido group. Since the attack by the 5-OH on the anomeric center is also from the opposite direction, a double inversion restores the anomeric center to the original β -configuration. In the end, the β -stereochemistry of furanoside **7** was well preserved during the rearrangement. The partial formation of small amounts of α -glucopyranosides in the cases of some secondary and tertiary alcohols can be attributed to the *in situ* acid-catalyzed anomerization of the initially formed β -glucopyranosides. When reactions mixtures of **5** with primary alcohols were left at room temperature for a prolonged time (several nights), we observed the formation of small amounts of α -pyranosides.

We also found support of this proposed mechanism from Jacquinet *et al*'s original publication,^[79] where the authors treated two free amino derivatives related to de-*N*-acetylated compound **6** or **7** with acids. It was observed that the transformations to the corresponding pyranoside were more difficult than with the *N*-acetylated derivatives **6** and **7**. This could be attributed primarily to the protonation of the amino groups; however, in our opinion, the absence of the acetamido group on the nitrogen to assist the opening of the furanoside is also a contributing factor. In addition, these authors used 60% acetic acid – water as solvent to transform compounds **6** and **7** to **10** in quantitative yield when heating. Consistent with our hypothesis, no other hydrolyzed or acetylated products were obtained, indicating that under these conditions water and acetate were not involved in the reaction.

To confirm the above hypothesis we investigated 1,2-*O*-isopropylidene- α -D-glucofuranose **11**, a furanoside that does not have an acetamido group to participate

during the reaction (Scheme 2.2). The reaction of **11** with methanol under anhydrous camphor-10-sulfonic acid (0.5 equiv) resulted in glycosylation that proceeded much more slowly (20 h), and the reaction gave the α/β mixtures of methyl furanosides and methyl pyranosides. These results are rationalized in terms of the absence in **11** of a neighboring acetamido group, and the accompanying acetamido group-assisted ring opening. For this reason the reaction proceeds more slowly, and since the 2-OH is not a participating group, there is no possibility to form cyclic intermediates to control the anomeric configuration of the resulted glycosides.



Scheme 2.2. The glycosidation of **11** with methanol in the presence of acids

2.5 Conclusion

In summary, we have discovered a new route to unprotected 2-acetamido-2-deoxy- β -D-glucopyranosides from *N*-acetyl-D-glucosamine, which proceeds via a furanoside oxazoline intermediate **5**. The method is well suited to large-scale work and for simple glycosides no chromatography steps are required. Glycosides with larger aglycones require a single chromatographic step. Since the product is a 2-acetamido-2-deoxy- β -D-glucopyranoside, this unprotected derivative is directly available for subsequent steps. The new methodology is mild and compatible with a wide range of functional groups, and has become the method of choice for the preparation of 2-acetamido-2-deoxy- β -D-glucopyranosides in our group.

Chapter Three

Concise Synthesis of Unusual 2-Acetamido-2-deoxy- β -hexopyranosides and Derivatives

3.1 Introduction

Although 2-amino-2-deoxyhexoses are widely distributed in a variety of organisms in nature, with the exception of plentiful and inexpensive D-glucosamine, most, including the more common D-galactosamine and D-mannosamine, are quite expensive. Others, especially 2,4-diamino-2,4-dideoxy sugars, are not yet commercially available. However, due to their significance in humans and other organisms, there is an increasing demand for many 2-amino-2-deoxysugars and their glycosides for biological or medical research.^[51] Therefore, efficient and large-scale chemical synthesis of these expensive 2-amino-2-deoxysugars and other derivatives in an expedient manner is a much sought after objective.

N-Acetyl-allosamine (AlINAc) is a rare naturally-occurring amino sugar. It is not commercially available, and was only found to exist in the disaccharide moiety of a powerful and selective chitinase inhibitor, allosamidin.^[80,81] D-Gulosamine, another rare aminosugar, was isolated from the antibiotic streptothricin F and streptolidin B,^[82] and its L-counterpart is a major constituent in another type of antibiotic, adenomycin.^[83] Most of the reported syntheses of D-allosamine and gulosamine start from inexpensive D-glucosamine.^[84-86] Traditionally, D-allosamine was prepared by a strategy involving selective 4,6-*O*-protection of D-glucosamine and followed by an inversion of configuration at C-3. Recently, a D-allosamine derivative was synthesized from a

trimesylate derivative of D-glucosamine by a double displacement at C-3 and C-6 position.^[88] Besides D-glucosamine as starting materials, D-allosamine was also prepared from a 4, 5-dihydroisoxazole in 5 steps.^[89]

The synthesis of D-gulosamine can be traced back to the 1960s when Tarasiejska and Jeanloz initially synthesized D-gulosamine by C-3 epimerization of D-galactosamine.^[90] Kuhn and Bister also achieved the synthesis of D-gulosamine starting from D-xylose.^[91] Another synthetic route to D-gulosamine was reported by Suami, via selective ring-opening of an epoxide intermediate from a readily accessible derivative of D-glucosamine.^[92] Unfortunately, all published synthetic routes suffer from either excessive length, poor yields, or the need for harsh reaction conditions. In particular, they are not suitable for large scale synthesis and especially for the synthesis of significant naturally occurring β -glycosides.

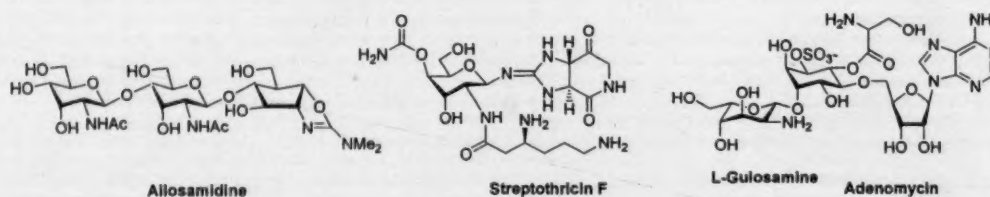


Figure 3.1. Representative examples of unusual 2-amino-2-deoxyhexosides in nature

N-Acetyl-galactosamine is a commonly-occurring constituent of natural oligosaccharides and glycoproteins, typically found α -linked to serine or threonine on *O*-glycans and β -linked in GSLs. In spite of its common existence in nature, D-galactosamine is quite expensive compared to the more abundant D-glucosamine. Consequently several chemical methods for the preparation of D-galactosamine have

been developed. One of these methods involves a double displacement on a dimesylated derivative of D-glucosamine, which was initially reported by Gross and co-workers.^[93] The double displacement was also performed on a trimesylate derivative of D-glucosamine to afford some D-galactosamine derivatives.^[88] Another common strategy involves selective protection of the *O*-3 and *O*-6 positions followed by a subsequent C-4-epimerisation, which could be achieved by intermolecular or intramolecular displacement. Using this strategy, Jacquinet and co-workers have prepared an α -glycoside of D-galactosamine from D-glucosamine by selective installation of pivaloyl groups at C-4 and C-6 positions.^[94] However, the synthesis of β -glycosides of D-galactosamine has not been reported yet by this method.

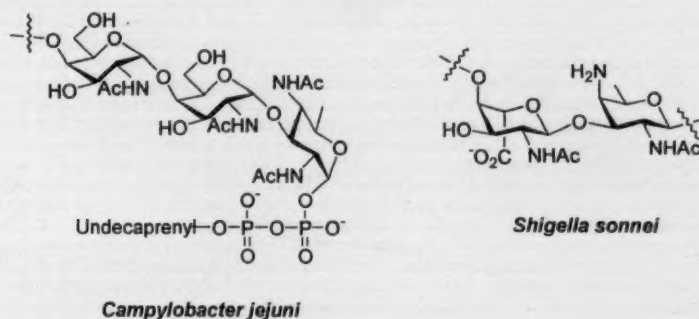


Figure 3.2. Representative examples of unusual 2,4-diamino-2,4,6-trideoxy sugars found in nature

Another important type of amino sugars are 2,4-diamino-2,4,6-trideoxyhexoses, which frequently occur as components of bacterial polysaccharides, as shown in Figure 3.2. 2,4-Diamino-2,4,6-trideoxy-D-glucose (also called as bacillosamine) was isolated from a *Bacillus subtilis* polysaccharide^[95] and the *N*-linked glycoprotein of *Campylobacter jejuni*.^[96] Its D-galacto stereoisomer is found to be a component of

capsular polysaccharide antigens of *Streptococcus pneumoniae* and *Shigella sonnei*.^[97,98] On the other hand, 2,4-diamino-2,4,6-trideoxyhexose with the D-*gulo* configuration can be recognized as a constituent of O-antigen lipopolysaccharides of gram-negative bacteria *Pseudomonas aeruginosa*.^[99,100] Due to their natural existence and biological importance, the chemical synthesis of these 2,4-diamino-2,4,6-trideoxy sugars has also been explored. However, many published syntheses of 2,4-diamino-2,4,6-trideoxy sugars involve lengthy and laborious reaction sequences. For instance, bacillosamine was synthesized from expensive benzyl 2-acetamido-2-deoxy- α -D-galactoside in 8-9 steps.^[101] the synthesis of methyl 2-acetamido-4-amino-2,4,6-trideoxy- β -D-galactoside was also reported but involved 17 steps from D-glucosamine.^[102]

In Chapter two, we have described a facile two-step approach to prepare unprotected 2-acetamido-2-deoxy- β -D-glucopyranosides via a furanose oxazoline donor **5** on a large scale. It is of interest to note that the furanose oxazoline has only one free hydroxyl group at the C-3 position, which can be readily available for functionalization or manipulation without additional protection (Figure 3.3). By combining this functionalization with glycosylation method we developed, a variety of functional groups could be rapidly introduced to provide 3-functionalized 2-acetamido-2-deoxy- β -D-glucopyranoside derivatives through the key intermediate **5**. These 3-functionalized derivatives would be valuable intermediates in the synthesis of some useful 2-acetamido-2-deoxy- β -D-glycosides. In this chapter, the introduction of mesyl, benzyl and lactic acid groups at C-3 of oxazoline **5** has been explored, and applied to the concise synthesis of unusual, but important, methyl 2-acetamido-2-deoxy- β -D-hexopyranosides as well as muramic acid, an important structural component of bacterial cell wall peptidoglycan.

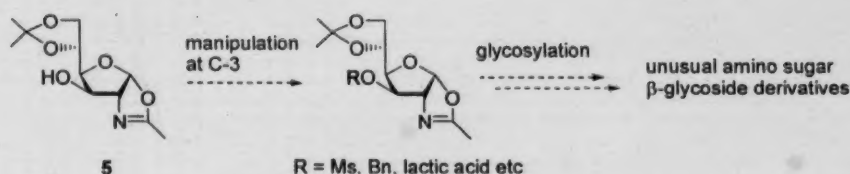


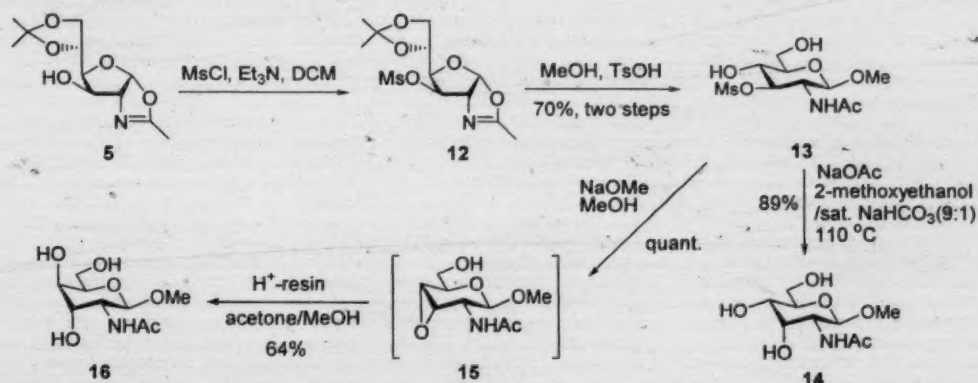
Figure 3.3. Proposed synthetic plan for unusual amino sugars via oxazoline **5**

3.2 Synthesis of methyl 2-acetamido-2-deoxy- β -D-allopyranoside and methyl 2-acetamido-2-deoxy- β -D-gulopyranoside

The first 3-functionalization of oxazoline **5** we explored was mesylation to be utilized in subsequent inversion of configuration. The mesylation of **5** was initially attempted by the standard conditions of mesyl chloride in dry pyridine but this was surprisingly unsuccessful. Then, $\text{CH}_2\text{Cl}_2/\text{Et}_3\text{N}$ mixture was used and it was found that the mesylation worked quite well. After simple extraction, the resulting crude **12** was directly used in the subsequent glycosylation. The glycosidation of **12** was carried out in the presence of 0.1 equivalent of *p*-toluenesulfonic acid in dry methanol to smoothly afford 3-mesylated β -glycoside **13** in 70% yield over 2 steps. The desired β -linkage of **13** is supported by a large $J_{1,2}$ coupling constant (8.4 Hz) in its ^1H -NMR spectra.

Next, the inversion of configuration at C-3 to give the *D-allo* configuration could be achieved by treatment with sodium acetate in a mixture of 2-methoxyethanol and sat. NaHCO_3 solution (9:1, v/v) at 110 $^\circ\text{C}$ for 18 h.^[6,7] Following this procedure, the 3-mesyl derivative **13** was smoothly converted to the desired methyl 2-acetamido-2-deoxy- β -D-allopyranoside **14** in 89% yield. The *allo* configuration of **14** is well established by the

small $J_{2,3}$ and $J_{3,4}$ coupling constants (2.8 Hz and 2.9 Hz) observed for H-3 in $^1\text{H-NMR}$ spectra.



Scheme 3.1. Synthesis of methyl 2-acetamido-2-deoxy- β -D-allopyranoside **14** and its *gulo* counterpart **16** from oxazoline **5**

Furthermore, treatment of **13** with 2M NaOMe in methanol for 4-5 hours at room temperature gave the 3,4-epoxide derivative **15** in almost quantitative yield. The synthesis of similar 3,4-epoxide derivatives of 2-amino-2-deoxyhexose have been reported previously by two different groups. A 3,4-epoxide derivative with the *galacto*-configuration was synthesized by Suami and coworkers from a 4-mesylated glucosamine derivative via harsh conditions using sodium hydride at 100°C .^[92] Zamojski and coworkers have also reported the synthesis of 3,4-epoxide derivative in only 53% yield from a 3-mesylated glucosamine derivative by a two-step procedure involving sodium hydride treatment as the second step.^[99] Compared to these previous syntheses, our procedure to the 3,4-epoxide derivative **13** is obviously very mild and efficient. By treatment with acidic resin (Amberlite IR-120) in a mixture of acetone and methanol, the epoxide **15** was transformed to give the desired methyl 2-acetamido-2-deoxy- β -D-

gulopyranoside **16** in 64% over two steps (from **13**) through a stereoselective diaxial ring opening reaction. The *gulo*-configuration of **16** is assigned based on the small value of the $J_{2,3}$ coupling constant (3.2 Hz) and the small value of the $J_{4,5}$ coupling constants (1.4 Hz). The assignment is also corroborated by a small $J_{3,4}$ coupling constant (3.5 Hz).

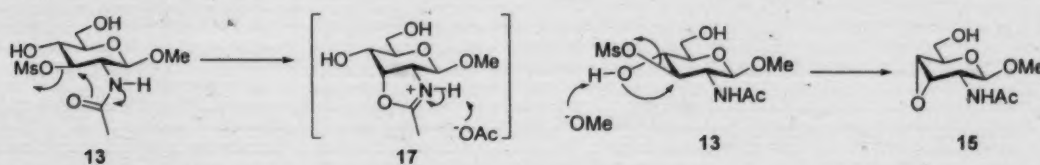
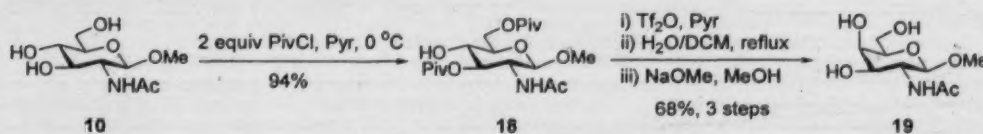


Figure 3.4. Intramolecular displacements on **13** by treatment with NaOAc and NaOMe respectively

The conversion of **13** to **14** has been assumed to proceed via an intramolecular displacement assisted by neighboring 2-acetamido group participation to give an intermediate **17**, which could be easily deprotonated by a weak base like sodium acetate and further hydrolysed in the reaction condition to provide the final compound **14** (Figure 3.4.). The formation of the 3, 4-epoxide of **15** could be rationalized by a deprotonation of adjacent 4-OH of **13** followed by another intramolecular displacement. When treated with a strong base like sodium methoxide, the deprotonation of 4-hydroxyl group of **13** proceeded much faster than the displacement assisted by neighboring acetamido group, thereby leading to the dominant epoxide product. These interesting findings will further facilitate the synthetic applications of **13** as a versatile building block.

3.3 Synthesis of methyl 2-acetamido-2-deoxy- β -D-galactopyranoside

Meanwhile, methyl 2-acetamido-2-deoxy- β -D-galactopyranoside **19** was also conveniently synthesized from oxazoline **5** as shown in Scheme 3.2. In Chapter two, we have prepared methyl 2-acetamido-2-deoxy- β -D-glucopyranoside **10** quantitatively from oxazoline **5** on a large scale without the need for chromatography.



Scheme 3.2. Synthesis of methyl 2-acetamido-2-deoxy- β -D-galactopyranoside **19**

In this context, compound **10** was treated with 2 equiv pivaloyl chloride in pyridine at 0°C according to Jacquinet's procedure to afford selectively a 3,6-*O*-pivaloyl protected D-glucosamine derivative **18** in excellent yield (94%).^[94] Next, inversion of configuration at C-4 was realized by a two-step one pot procedure: a triflation followed by a $\text{S}_{\text{N}}2$ -displacement reaction. The triflation of **18** was carried out with triflic anhydride in pyridine at 0°C , and water was subsequently added. The resulting mixture was heated for several hours to promote the inversion. During this process, it is noteworthy that 3-pivaloyl group actually migrated to the C-4 position by an intramolecular displacement and exposed a free hydroxyl group at C-3 position to give a 4,6-*O*-pivaloyl protected intermediate **20** (Figure 3.5). The intermediate **20** was then subjected to transesterification conditions and all these three steps were performed in a one-pot procedure to give methyl 2-acetamido-2-deoxy- β -D-galactopyranoside **19** in 68% overall

yield. The desired *galacto*-configuration of **19** is well supported by the value of $J_{3,4}$ coupling constant (3.3 Hz) and the small value of the $J_{4,5}$ coupling constant (0.6 Hz).

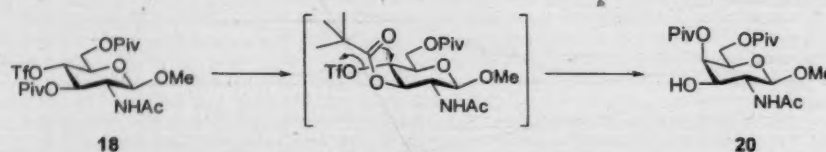


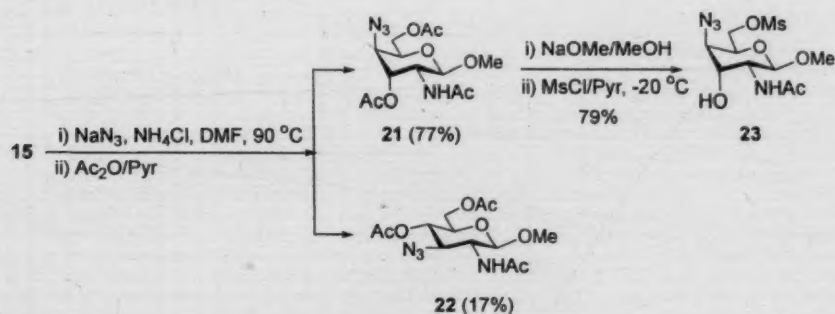
Figure 3.5. Postulated mechanism of pivaloyl group migration from C-3 to C-4

3.4 Synthesis of methyl 2-acetamido-4-amino-2,4,6-trideoxy- β -D-gulopyranoside

The synthesis of the 2,4-diamino-2,4,6-trideoxy-D-gulopyranose and derivatives was only previously reported by Banaszek *et al.* via a 3,4-epoxide intermediate.^[99,100] However, their synthesis is not efficient due to the laborious reaction sequence and moderate yield of 3,4-epoxide formation. In our previous synthesis, epoxide **15** was quantitatively prepared in large quantity from D-GlcNAc via a short sequence. This compound was successfully employed to prepare methyl 2-acetamido-2-deoxy- β -D-gulopyranoside **16** through a stereoselective diaxial ring opening. Herein, the epoxide **15** was further applied to the efficient synthesis of 2,4-diamino-2,4,6-trideoxyhexoses **26** with the D-*gulo*-configuration.

The ring opening reaction of epoxide **15** is described in Scheme 3.3. By treatment with NaN_3 in the presence of NH_4Cl in dry DMF at 90 °C, the epoxide **15** was converted into two isomers: the one with D-*gulo*-configuration was the major product (77%) and the other, with D-*gluco*-configuration, was the minor product (17%). Unfortunately, it was found that these two isomers were extremely difficult to separate, even by silica gel

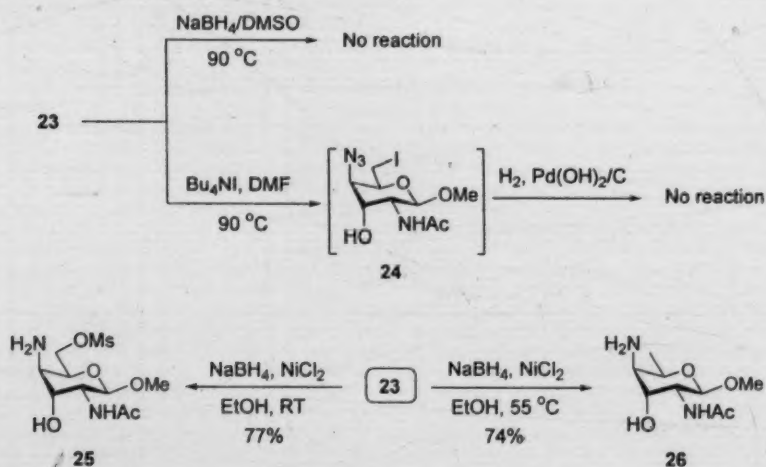
chromatography. To circumvent this problem, an acetylation step was applied and then two pure diacetate derivatives **21** and **22** could be isolated. Deacetylation of **21** followed by treatment with 1 equivalent of mesyl chloride in dry pyridine at $-20\text{ }^{\circ}\text{C}$ selectively afforded the desired 6-mesylate **23** in 79% yield, which was then subjected to the next deoxygenation step (Scheme 3.4).



Scheme 3.3. Ring-opening of an epoxide **15**

The 6-deoxygenation of **23** was initially attempted with NaBH_4 in DMSO at elevated temperature, but unfortunately no reaction was observed. The 6-mesylate group of **23** was next replaced by iodine to give a 6-iodo derivative **24**. However, our attempts at dehalogenation of **24** by hydrogenation over $\text{Pd}(\text{OH})_2/\text{C}$ were unsuccessful. An alternative reductive condition of $\text{NaBH}_4/\text{NiCl}_2$ in EtOH, was then employed in our synthesis (Scheme 3.4). These conditions have been effectively utilized in dehalogenation and azido reduction.^[103-105] However, it was found that, by treating with $\text{NaBH}_4/\text{NiCl}_2$ in EtOH at room temperature, only the azide was reduced to give a 4-amino derivative **25** in 77%; and no corresponding 6-deoxygenated product was obtained. Fortunately, when conducting the reaction at elevated temperature ($50\text{--}60\text{ }^{\circ}\text{C}$), both the azido reduction and deoxygenation of **23** proceeded concurrently to give the desired 2,4-diamino-2,4,6-

trideoxy compound **26** with the *D-gulo*-configuration in 74% yield. The *D-gulo* configurations of **26** was unambiguously confirmed by three small $J_{2,3}$, $J_{3,4}$ and $J_{4,5}$ coupling constants (3.2 Hz, 3.2 Hz, 1.8 Hz).



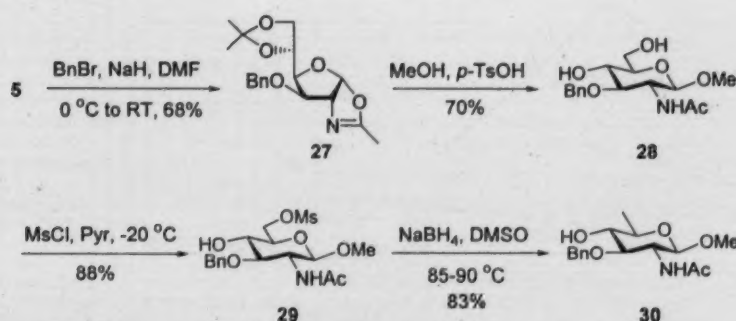
Scheme 3.4. Studies on reductions of **23** to **26**

3.5 Synthesis of methyl 2-acetamido-4-amino-2,4,6-trideoxy- β -D-galactopyranoside

Although syntheses of 2,4-diamino-2,4,6-trideoxy-D-galactopyranose derivatives have been reported by several research groups,^[106,107] all of the reported synthetic routes are fairly lengthy and laborious. For example, the synthesis of target compound methyl 2,4-diamino-2,4,6-trideoxy- β -D-galactopyranoside **36** has been reported in 17 steps by Medgyes *et al.* starting from D-glucosamine.^[102] The inefficiency of the reported synthesis prompted us to exploit our oxazoline method in the synthesis of this compound.

Our synthesis of methyl 2-acetamido-4-amino-2,4,6-trideoxy- β -D-galactopyranoside **36** starting from oxazoline **5** is depicted in Scheme 3.5. Before manipulations at the C-4 and C-6 positions, the 3-OH had to be blocked with a suitable protecting group.

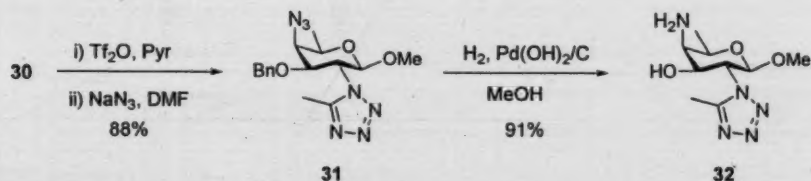
Treatment with benzyl bromide in the presence of NaH in dry DMF gave the desired 3-*O*-benzylated oxazoline **27**. Glycosidation of **27** with methanol was carried out in the presence of *p*-toluenesulfonic acid to afford the desired methyl β -glycoside **28** in 70% yield. The β -configuration was determined by a $J_{1,2}$ coupling constant of 8.4 Hz. Selective mesylation at C-6 was achieved in 88% yield by treating **28** with 1 equivalent of mesyl chloride at -20°C to give **29**. Subsequent reduction of **29** with NaBH_4 in dry DMSO at elevated temperature ($85\text{--}90^\circ\text{C}$) furnished the 6-deoxy derivative **30** in 83% yield.



Scheme 3.5. Synthesis of 6-deoxy derivative **30** from oxazoline **5**

The introduction of an azido functionality at the C-4 position through an inversion of configuration was accomplished by triflation and displacement with an azido group. Triflation of the 4-OH of **30** was first attempted by reacting with 2 equivalents of triflic anhydride in pyridine at 0°C , conditions that were recently employed by Imperiali's group in the introduction of an azido group at C-4 of benzyl 2-acetamido-2-deoxy-3,6-*O*-dibenzoyl- β -D-galactopyranoside without affecting the 2-acetamido group.^[101] However, to our surprise, a 2-tetrazole containing galactosamine derivative **31** was exclusively obtained, in a high yield of 88%, after treatment with sodium azide in DMF at RT. The

derivative **31** was still obtained as a sole product even if triflation was carried out at lower temperature $-30\text{ }^{\circ}\text{C}$. The presence of the tetrazole moiety in **31** was determined by HRMS, ^1H and ^{13}C NMR experiments, and elemental analysis. We noted from ^{13}C NMR that the chemical shift of the carbonyl-carbon in **30** was shifted from 170 ppm to 154.2 ppm in **31**, which is within the typical range (150-160 ppm) of tetrazole carbons.^[110] This assignment was also corroborated by ^1H NMR spectrum of **31**, in which a downfield shift value at 2.54 ppm corresponding to a tetrazole- CH_3 was also found. The azide functionality of **31** was selectively reduced under standard hydrogenation conditions without affecting the 2-tetrazole moiety, affording **32** in 91% yield.



Scheme 3.6. Synthesis of a tetrazole-containing derivative **32** from **30**

Explanation of the above experimental results, and the mechanism of 2-tetrazole formation in **31** are described in Figure 3.6. The 2-tetrazole moiety was presumably derived from the reaction of the 2-acetamido group with a second equivalent of triflic anhydride. Upon treatment with an excess amount of triflic anhydride, a 2-imidodyl triflate intermediate **33** was generated and then attacked by azide to afford intermediate **34**, which rapidly underwent an intramolecular cyclization to afford a 2-tetrazole derivative **31**. The formation of 2-tetrazole in our substrate **30**, but not Imperiali's, substrate is presumably due to the enhanced reactivity of our substrate. Compared to the Imperiali's substrate, which has two electron-withdrawing groups like benzoyl group, our

substrate **30** is conceivably much more reactive because of the presence of 6-deoxygenation and an electron-donating benzyl group at C-3.

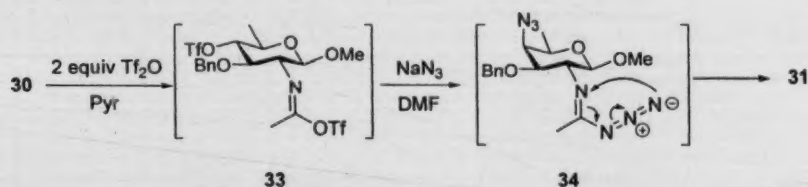
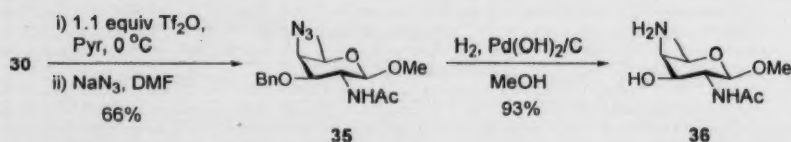


Figure 3.6. Postulated mechanism of tetrazole formation on **30**

To avoid the unwanted formation of a 2-tetrazole moiety, compound **30** was treated with a nearly stoichiometric amount of triflic anhydride (1.1 equiv) in pyridine at 0 °C. Now, to our satisfaction, the desired compound **35** was successfully obtained as the major product in 66% yield after treatment with sodium azide in DMF at RT. The *D-galacto* configuration was clearly indicated by the value of $J_{3,4}$ (3.6 Hz) coupling constant and a small $J_{4,5}$ (0.9 Hz) coupling constant. Finally, by a standard hydrogenation over $\text{Pd}(\text{OH})_2/\text{C}$ in methanol, the reduction of the azido group of **35** was concomitantly achieved with deprotection of the 3-*O*-benzyl group to provide the target compound **36** in 93% yield. Both the ^1H and ^{13}C NMR data are in agreement with those reported by Medgyes *et al.*^[102] The observed optical rotation value of **36** is also close to that in the literature ($[\alpha]_{\text{D}} -10.8^\circ$ (*c* 0.91, MeOH); lit.^[102] ($[\alpha]_{\text{D}} -8.7^\circ$ (*c* 1.0, MeOH))). Overall, by employing our oxazoline method, methyl 2,4-diamino-2,4,6-trideoxy- β -*D*-galactopyranoside **36** was conveniently synthesized from inexpensive *D*-GlcNAc in only 7 steps compared to the 17 steps in the literature.^[102]



Scheme 3.7. Synthesis of methyl 2-acetamido-4-amino-2,4,6-trideoxy- β -D-galactopyranoside **36**

It is noteworthy that a tetrazole moiety formed at the acetamido position of carbohydrates is quite interesting. Although a number of tetrazole derivatives have been prepared from various starting materials using a variety of methods such as $\text{Ti}_2\text{O}/\text{NaN}_3$,^[108] $\text{PPh}_3/\text{DEAD}/\text{TMSN}_3$ ^[109] and $\text{SnCl}_2/\text{TMSN}_3$,^[110] few examples of tetrazole containing carbohydrates have been reported before. Considering the medical importance of the tetrazole moiety and the common occurrence of acetamido group on many important carbohydrates (such as 2-acetamido-2-deoxyhexoses, sialic acid, etc.), our approach to converting the acetamido group to a tetrazole group by using $\text{Ti}_2\text{O}/\text{NaN}_3$ might probably provide a new strategy for rapidly modifying carbohydrates with the objective of developing novel inhibitors, probes and other carbohydrate based drugs.

3.6 Synthesis of *N*-acetylmuramic acid and muramylpeptides

3.6.1 Introduction

N-Acetylmuramic acid (MurNAc) is the major constituent of the repeating structure that constitutes the two dimensional array of bacterial cell wall peptidoglycan, known as murein. Structurally, the murein is a biopolymer consisting of alternating β -(1 $\rightarrow$ 4) linked disaccharide units of *N*-acetyl-glucosamine (NAG) and *N*-acetyl-muramic acid (NAM), cross-linked via peptides appended to the muramyl moiety. Adjacent peptide chains are cross-linked by the transpeptidase enzymes of cell wall biosynthesis to provide the cell wall polymer with the rigid structural integrity required by the bacterium.

[111]

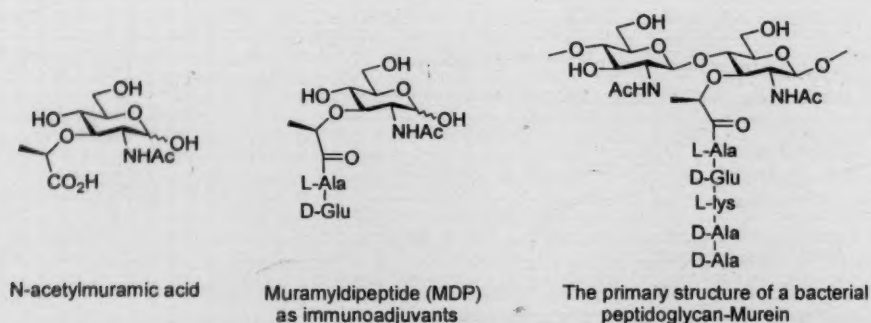


Figure 3.7. The structures of MurNAc, MDP and bacterial cell wall peptidoglycan

Muramylpeptides were previously studied for their immunoadjuvant activities and stimulation of non-specific resistance against bacterial, viral, and parasite infections, as well as anticancer properties.^[112] For example, muramyl dipeptide (MDP), namely *N*-acetylmuramyl-L-alanyl-D-isoglutamine, was intensively investigated as the smallest immunoadjuvant capable of replacing whole mycobacteria in Freund's complete adjuvant. Usually, MDP is the minimum structure required for immunoadjuvant

activities. However, further investigations have shown that disaccharides or oligosaccharides linked to peptides have higher immunoadjuvant activities than those of MDP.^[113] Recently, studies on UDP-MurNAc derivatives and precursors of lipid I and II in the biosynthesis of bacterial peptidoglycan have re-emerged as important targets for developing new antibiotics to fight against antimicrobial resistance.^[114-119] The need for new antibiotics to treat resistant infections has led to a resurgence of interest in bacterial enzyme involving in peptidoglycan biosynthesis.

Several chemical syntheses of MurNAc and its peptide derivatives have been described in the literature.^[120-128] For instant, Schmidt *et al.* has synthesized MurNAc pentapeptides from a 2-azido-2-deoxy-D- β -glucoside derivative.^[126] Recently, Mobashery reported a synthetic route to the methyl β -glycoside of MurNAc and muramyl pentapeptide derivatives using the traditional oxazoline method.^[127,128] Several precursors and fluorinated analogues of MurNAc have also been prepared.^[127] Most of these syntheses employed a common strategy involving 4,6-protection of glucosamine followed by introduction of a lactate moiety. This strategy has also been utilized in the preparation of Lipid I and II precursors—muramylpentapeptides bearing a free anomeric hydroxyl group.^[116,117] An alternative strategy was reported by Brossmer to synthesize efficiently a MurNAc with a free anomeric group via a 5,6-*O*-isopropylidene furanose oxazoline **5** from inexpensive D-GlcNAc.^[124] But, so far, there have been no related reports on the synthesis of corresponding β -glycosides.

Besides MurNAc monosaccharide derivatives, the synthesis of *N*-acetylglucosamine (NAG)- β -(1 $\rightarrow$ 4)-*N*-acetyl-muramic acid (NAM) disaccharide repeating unit of bacterial cell wall peptidoglycans has also received extensive attention.^[125,128] The

synthesis of the NAG-NAM disaccharide unit is a significant challenge due to the formidable glycosylation problems associated with the MurNAc moiety: the least nucleophilic 4-hydroxyl group, an easily occurring intramolecular lactonization at the 4-hydroxyl position when the lactate is protected as an ester, and the possible racemization of lactate residues. To tackle these problems, several kinds of glycosyl donors including oxazoline,^[121] glycosyl halides,^[117,122,125] glycosyl imidate,^[127,128] and several *N*-protected groups such as phthalimido,^[122] Troc^[125] and dimethylmaleoyl (DMM)^[127,128] have been investigated, but none of them offers attractive solutions with regard to numbers of synthetic steps, and preparation in quantity. For example, some glycosyl donors, such as oxazolines and imidates gave poor yields (40-50%) even in glycosylations using a building block without a lactate residue. To prevent unwanted intramolecular cyclization, the lactate residue usually needs to be orthogonally protected by other groups such as phenylsulfonyl^[129] or by an alanine residue,^[117,125] and this undoubtedly decreases the coupling efficiency.

Considering the universal existence of the MurNAc moiety as a β -linkage in all bacterial cell wall peptidoglycans, a large scale and efficient synthesis of MurNAc and its derivatives with a natural β -linkage is highly desirable. This would provide convenient access to cell wall biosynthetic precursors or substrates for antibiotic binding studies. In this chapter we have demonstrated the extension of our furanose oxazoline method to the expedient synthesis of unusual 2-amino-2-deoxy hexosugars and derivatives. Herein, we further extend this oxazoline method to the efficient synthesis of MurNAc and especially its β -glycosides, as shown in Figure 3.8. An efficient elaboration of the core repeating NAG-NAM disaccharide unit bearing a β -(1 $\rightarrow$ 4) linkage is also explored in this context.

With the MurNAc monosaccharide β -glycoside and disaccharide unit in hand, both could be easily coupled with suitable oligopeptides via the lactic acid moiety to provide immunoadjuvants and precursors for bacterial cell wall biosynthesis studies.

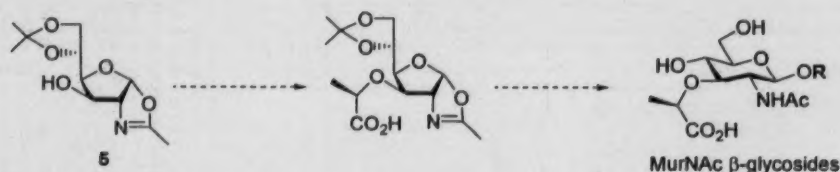
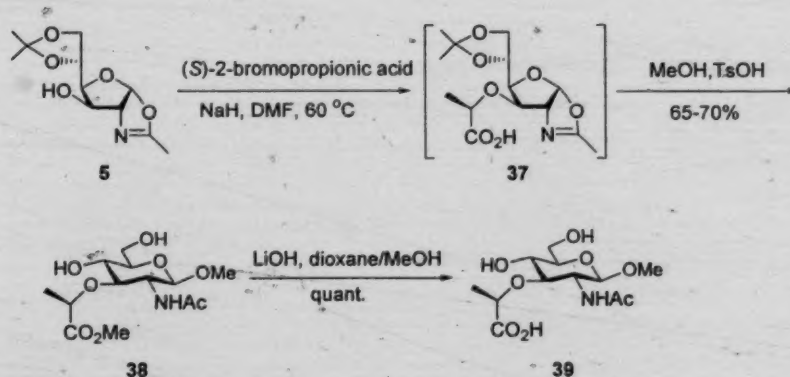


Figure 3.8. Synthetic plan of MurNAc β -glycosides from oxazoline **5**

3.6.2 Synthesis of MurNAc methyl β -D-glycoside

As discussed above, the synthesis of β -glycosides of MurNAc started from the furanose oxazoline **5**, available on a large scale from D-GlcNAc (over 20 grams in one step without chromatography). By treating **5** with sodium hydride followed by adding (*S*)-2-bromopropionic acid in dry DMF, lactic acid was successfully introduced at the C-3 position of **5** without racemization to afford intermediate **37**, the structure of which was determined by ¹H-NMR spectroscopy. Since **37** was found to be partially hydrolysed during the work-up procedure, a crude separation by a short silica pad was applied to provide a crude compound that could be employed in the next glycosylation. The glycosylation was then conducted in dry methanol in the presence of *p*-toluenesulfonic acid, affording the desired methyl β -glycopyranoside of MurNAc methyl ester derivative **38** in 67% yield over two steps. The β -linkage of **38** was clearly confirmed by a large $J_{1,2}$ coupling constant (8.5 Hz). Finally, the methyl ester group was smoothly hydrolyzed by 1M lithium hydroxide in a mixture of dioxane and methanol to afford the methyl β -

glycopyranoside of MurNAc **39** in almost quantitative yield. No racemization product was observed during the deesterification. It should be mentioned that this short synthesis has been scaled up to multi-gram quantities and this approach could also be extended to other β -glycosides.



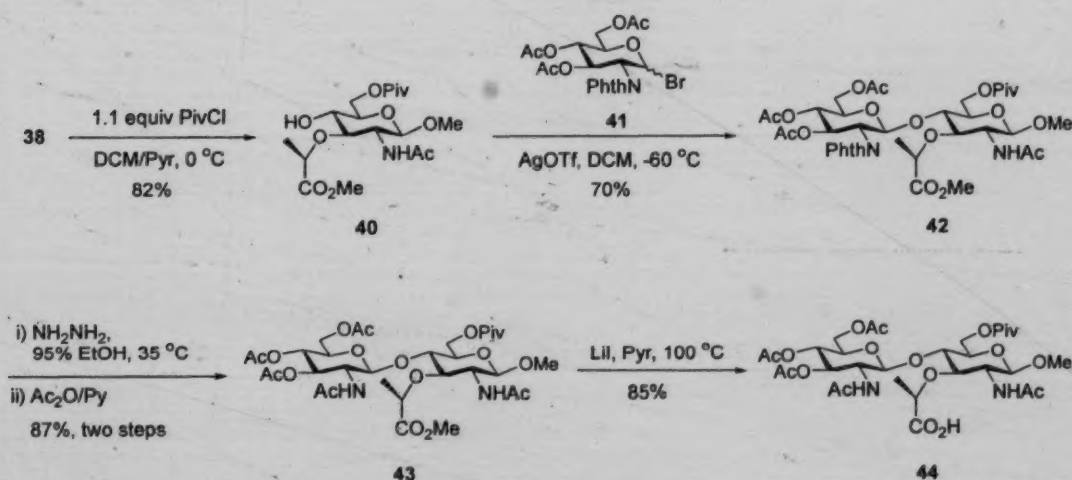
Scheme 3.8. Synthesis of MurNAc methyl β -glycoside **39**

3.6.3 Synthesis of a methyl β -D-GlcNAc-(1 $\rightarrow$ 4)- β -D-MurNAc disaccharide unit

When MurNAc methyl β -glycoside was readily accessible from oxazoline **5** in two steps, the synthesis of the repeating β -(1 $\rightarrow$ 4) NAG-NAM disaccharide unit was then conducted. To facilitate this disaccharide unit synthesis, the MurNAc glycoside derivative **38** was chosen as the acceptor in our direct glycosylation without any further manipulation of the lactate residue of MurNAc. However, there are few successful examples for direct glycosylation, and only a β -glycosyl chloride has been previously employed as the donor to give the product in a reasonable yield.^[122] The more common α -glycosyl bromide donor has been reported to provide a complicated mixture of products at room temperature. Considering the ease of accessibility of 2-deoxy-2-*N*-

phthalimido protected α -glycosyl bromide donor, we thus decided to re-examine its direct glycosidation by acceptor **38**.

Compound **38** was selectively protected by treatment with 1.1 equivalent of pivaloyl chloride in pyridine at 0 °C to give the 6-*O*-pivaloyl protected derivative **40** in 82% yield. Then, the direct glycosylation between the glycosyl bromide donor **41** and the acceptor **38** was re-investigated. Since the glycosyl bromide donor is more reactive than its chloride counterpart, the direct glycosylation was examined at low temperature using silver triflate as the promoter. Fortunately, we found that glycosylation yields were increased by decreased glycosylation temperature, and the unwanted intramolecular cyclization product could be effectively suppressed at low temperature. For example, a satisfactory 70% yield of disaccharide **42** was obtained as the sole product at -60 °C, and no lactone side-product was observed. The desired β -(1 $\rightarrow$ 4) linkage of **42** was ambiguously established by a two large $J_{1,2}$ (7.8 Hz) and $J_{1',2'}$ (8.4 Hz) coupling constants. This direct glycosylation has already been scaled to gram quantity.



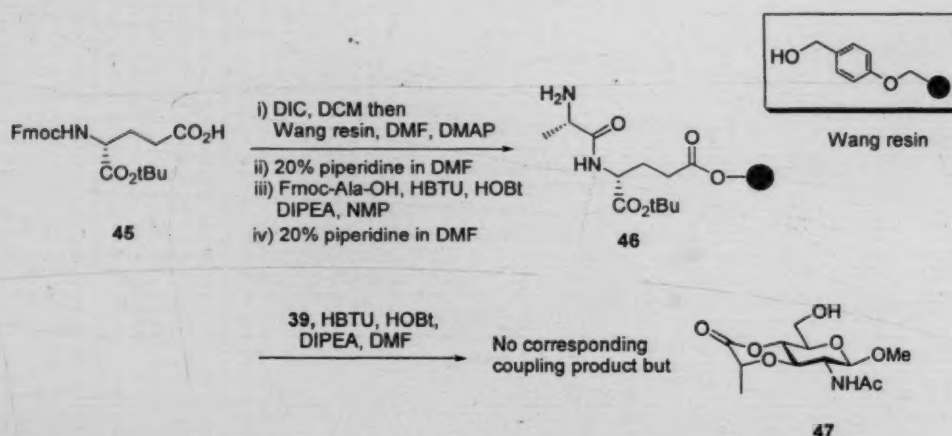
Scheme 3.9. Synthesis of a disaccharide unit **44** from **38**

Next, to avoid the formation of a stable hydrazide derivative from the ester residue during the removal of the phthalimido group, **42** was treated with dilute hydrazine in EtOH at 35 °C,^[130] which selectively cleaved the phthalimido group without removal of the methyl ester residue. In this context, the selective removal of the phthalimido group was performed together with a subsequent re-acetylation in one-pot manner to afford the desired compound **43** in 87% overall yield. The methyl ester in **43** was then selectively removed without deacetylation by treatment with lithium iodide in dry pyridine at 100 °C^[131] to provide the desired carboxylic acid derivative **44** in 85% yield, which can be directly used in the next coupling with suitable peptides.

3.6.4 Preliminary synthetic efforts of muramylpeptides

Having compounds **39** and **44** in hand, the synthesis of the corresponding muramylpeptides would logically become our next research focus. In this context, the coupling of **39** with a dipeptide (L-Ala-D-Glu) to a muramyldipeptide (MDP) was preliminarily investigated by using a solid-phase synthetic approach. The synthesis of dipeptide (L-Ala-D-Glu) on Wang resin by using standard Fmoc chemistry is shown in Scheme 3.10. First, the Fmoc-D-Glu-OtBu **45** was loaded onto Wang resin, by using symmetric anhydride method.^[183] The removal of Fmoc group by 20% piperidine in DMF and subsequent coupling with the second peptide Fmoc-Ala-OH using coupling reagents HBTU and HOBt gave the desired dipeptide **46** on resin. After the cleavage of terminal Fmoc group again, the coupling with muramic acid **39** was examined in the presence of HBTU and HOBt coupling reagents. However, no desired muramylpeptides but an

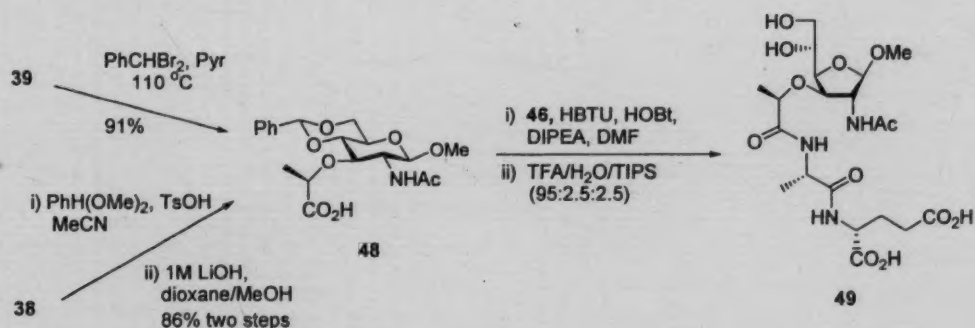
intramolecular cyclization side product **47** was observed. Similar lactone side products were also reported in previous literature.^[122,125]



Scheme 3.10. Synthetic studies of muramyl dipeptide by solid phase approach

To prevent the unwanted intramolecular cyclization, 4- and 6-OH of **39** were then protected by a benzylidene acetal before its coupling with dipeptide **46**. Treatment of **39** with α, α' -dibromotoluene in pyridine at 110 °C gave the desired 4,6-*O*-benzylidene protected muramic acid **48** in 91% yield. Compound **48** could be also prepared from **38** in 86% yield by the treatment with benzaldehyde dimethyl acetal and a catalytic amount of *p*-TsOH in acetonitrile followed by saponification with 1M LiOH in dioxane and methanol. The ¹H NMR spectrum clearly showed the presence of the β -pyranoside of **48**. Compound **48** was then successfully coupled with dipeptide **46** on resin using the same coupling conditions as above. The cleavage of muramyl dipeptide from the resin and the removal of protecting groups could be realized by treatment with TFA/water/TIPS (95:2.5:2.5). Unfortunately, the β -furanoside **49** was obtained, in contrast to our expectation. The β -furanoside configuration of **49** was assigned by a series of NMR

experiments including ^1H , ^{13}C , GHMQC and GHMBC. The coupling constants of H-3 ($J = 4.9\text{ Hz}$, $J = <1\text{ Hz}$) indicated the presence of furanoside instead of pyranoside in **49**, because normally there are two large coupling constants for H-3 in pyranosides. The β -anomeric configuration of **49** was determined by a small $J_{1,2}$ value between H-1 and H-2. Both coupling constants of H-1 and H-3 in **49** are very close to those in a similar furanoside **7** described in Chapter two. The structure was also corroborated by a downfield chemical shift (108.3 ppm) of the anomeric carbon in the ^{13}C -NMR spectrum, which corresponds to 1,2-trans furanosides. The unexpected β -furanoside **49** was presumably formed from a rearrangement that occurred during the extended reaction time, which was required for the cleavage of muramyldipeptide from the resin. To circumvent this problem, a more acid-labile resin such as, 2-chlorotrityl chloride resin, should be employed in the future synthesis of muramylpeptides.



Scheme 3.11. Synthesis of muramyldipeptide **49** from **38** and **39**

3.7 Conclusions

In summary, the extension of our facile oxazoline method to the concise synthesis of some unusual, but important, 2-amino-2-deoxy-sugars and derivatives has been performed. This work took advantage of oxazoline **5** as a key and versatile intermediate by manipulation or functionalization at the C-3 position. Through this strategy, three methyl 2-acetamido-2-deoxy- β -D-hexopyranosides (*galacto*-, *allo*-, and *gulo*-configurations) were prepared from oxazoline **5** in 3-4 sequences over 10 grams, and two methyl 2-acetamido-2,4-diamino-2,4,6-trideoxy- β -D-hexopyranosides (*galacto*- and *gulo*-configurations) were readily obtained in short sequences.

Besides, our facile oxazoline method was also successfully applied in the expedient synthesis of biologically important muramic acid and a repeating β -D-GlcNAc (1 $\rightarrow$ 4)- β -D-MurNAc disaccharide unit of bacterial cell wall peptidoglycan. The resulting muramic acid was further coupled with a dipeptide (L-Ala-D-Glu) by solid-phase synthesis to afford a muramyl dipeptide isomer. This extension provides relatively short and practical access to some unusual and expensive 2-acetamido-2-deoxy- β -D-hexopyranoside derivatives from inexpensive 2-acetamido-2-deoxy-D-glucosamine.

Chapter Four

Synthesis of Sphingolipids via Olefin Cross Metathesis and Asymmetric Aldol Condensation

4.1 Introduction

Sphingosines, sphinganine (dihydrosphingosines) and phytosphingosines (saturated and unsaturated) are common long-chain structural constituents of sphingolipids (Figure 4.1). Although particularly abundant in mammalian cells, they are also present in bacteria, fungi, plants and marine organisms. In addition to their structural functions, they are also involved in various biological activities, and play critical roles in many physiological processes, including modulation of immune responses, signaling, and cellular recognition.^[132] Sphingosine and sphinganine both strongly inhibit protein kinase C^[133] and their ceramide derivatives are potent stimulators of the mammalian immune system.^[134] Phytosphingosine is a potential heat stress signal in yeast cells,^[135] and some of its derivatives exhibit important physiological activities. For example, KRN7000, an α -galactosylphytosphingosine derivative isolated from a marine sponge, was found to be a powerful immunostimulant of natural killer T (NKT) cells as it is an excellent ligand for the CD1d protein on certain antigen presenting cells.^[22]

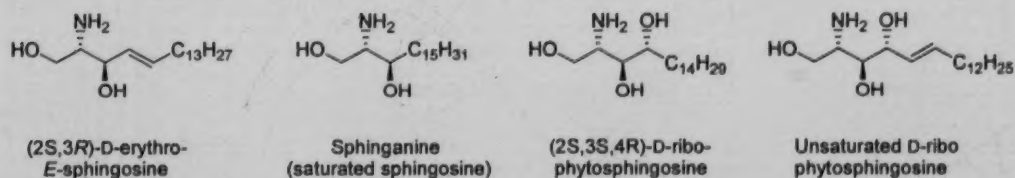


Figure 4.1 The chemical structures of major types of naturally occurring sphingolipids

Due to their biological significance, as well as the complication of isolation from natural sources in homogeneous form, a great deal of effort has been devoted to synthetic studies of this class of compounds, and a wide variety of methods have thus been developed.^[136,137] Based on the elaboration of stereochemistry of the amino-alcohol backbone, most published sphingolipid syntheses can be categorized into two major strategies: a chiral pool approach (carbohydrates and amino acids as starting materials) and an asymmetric induction approach (including Sharpless asymmetric epoxidation, asymmetric aldol reaction through chiral auxiliaries and catalysts). Among sphingolipids, *E*-sphingosine with C-18 is the most common target of synthetic interest. The synthesis of *E*-sphingosine can also be classified based on the formation of the *E*-double bond. The *E*-double bond of sphingosine has been constructed by many methods, such as anti-hydrogenation of an alkyne, Wittig reaction, Horner-Wadsworth-Emmons (HWE) reaction, S_N2' reaction by Grignard reagents and anti-elimination of β -hydroxyselenide.

Recently, a new strategy, olefin cross metathesis (OCM), was introduced to construct *E*-configured sphingosine. Olefin metathesis, including ring-closing metathesis and cross metathesis, has been widely applied in carbohydrate chemistry in the past decade.^[138] Our group first designed and synthesized a truncated version of sphingosine which preserves the important stereochemical and structural features of naturally occurring sphingosine on a large scale (Scheme 4.2).^[139] This versatile truncated structure can be applied to the synthesis of various sphingosine analogues and derivatives via several methods, including olefin cross metathesis (OCM). Indeed, ganglioside synthesis via an OCM strategy has previously been explored by our group but the yield was unsatisfactory.^[139b] The syntheses of *E*-sphingosine by OCM in good to high yields were

reported by the Somfai group^[140a] and the Basu group^[140b] respectively, starting from similar truncated structures. Besides, several other sphingosine derivatives, such as azido-sphingosine,^[140c] sphingosine-1-phosphate^[140d] as well as fluorescent labelled ceramides were recently synthesized via OCM strategies.^[140e-d] So far, olefin cross metathesis (OCM) has become a powerful chain elongation approach in the synthesis of *E*-configured sphingosines and analogues.

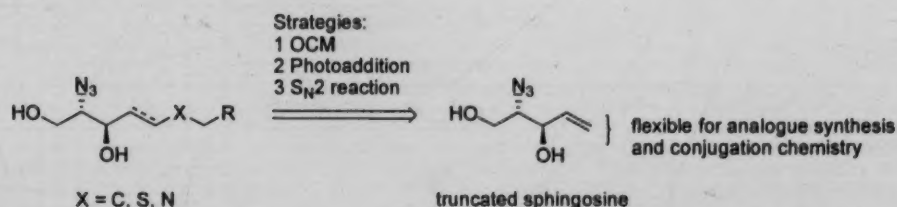


Figure 4.2 Our retrosynthesis of sphingosine analogues and derivatives from truncated sphingosine

In spite of numerous syntheses of sphingosine and other sphingolipids currently available, more efficient synthetic approaches continue to be needed considering the demand for glycosphingosines in biological or medical studies. In particular, as a part of our efforts to develop novel α -galactosylceramide and ganglioside-based immunotherapies, we have considerable interest in developing new versatile methods, which are highly flexible in the synthesis of sphingolipid stereoisomers, analogues and derivatives, as well as for the purpose of conjugation chemistry.

4.2 Synthesis of C-18-phytosphingosines via olefin cross metathesis

Like *E*-sphingosine, the chemical synthesis of phytosphingosines has also been achieved by a variety of methods mainly including a chiral pool approach and asymmetric synthesis.^[141,142] However, most approaches often result in minor amount of diastereomers that need to be separated, or require lengthy routes to reach the target compound.^[142a] Moreover, these approaches lack flexibility to access phytosphingosine stereoisomers and analogue synthesis. On the other hand, although the largest group of plant GSLs contains a saturated phytosphingosine moiety, there are also many examples of unsaturated *E*-phytosphingosines with a 5,6-double bond found in plants and even marine animals.^[11] These unsaturated 5(*E*)-phytosphingosines also exhibited a variety of significant biological and physiological properties. However, almost all current synthetic efforts have focused mainly on saturated phytosphingosines and there are few reported examples of the synthesis of unsaturated *E*-phytosphingosine. Herein, we describe a new general and flexible approach, which allows us to prepare not only both saturated and unsaturated phytosphingosines, but also a series of their stereoisomers and analogues.

4.2.1 Retrosynthetic analysis

To achieve the above goals, an olefin cross metathesis (OCM) strategy was introduced into our synthesis. As outlined in the retrosynthetic plan in Figure 4.3, C-18-phytosphingosines including stereoisomers, are disconnected to truncated C-6-phytosphingosine structures **I**, which retain the same stereochemical features of the targeted compounds, and 1-tetradecene. Therefore, in principle, various lipid chains with different lengths or functional groups can be easily introduced for the preparation of a series of phytosphingosine analogues and derivatives using this strategy. However, the

application of OCM strategies in sphingolipids has concentrated on *E*-sphingosine. The synthesis of phytosphingosine via OCM reactions is less frequent and only three related reports have appeared in the literature. Han first completed the synthesis of *L*-lyxo and *L*-arabino-phytosphingosines by a OCM strategy, but the natural *D*-ribo-phytosphingosine has not yet been achieved.^[143a] The synthesis of *D*-ribo-phytosphingosine was recently realized by OCM reactions, but the synthesis of the corresponding truncated structure is not efficient due to difficult diastereomers separations.^[143b] More recently, Lin reported a synthetic route to phytosphingosines from *D*-lyxose, via OCM reactions but the yields are only 49-60%.^[143c] Moreover, all these reported synthetic approaches focused on a single phytosphingosine only and thus are not applicable to the synthesis of other diastereoisomers.

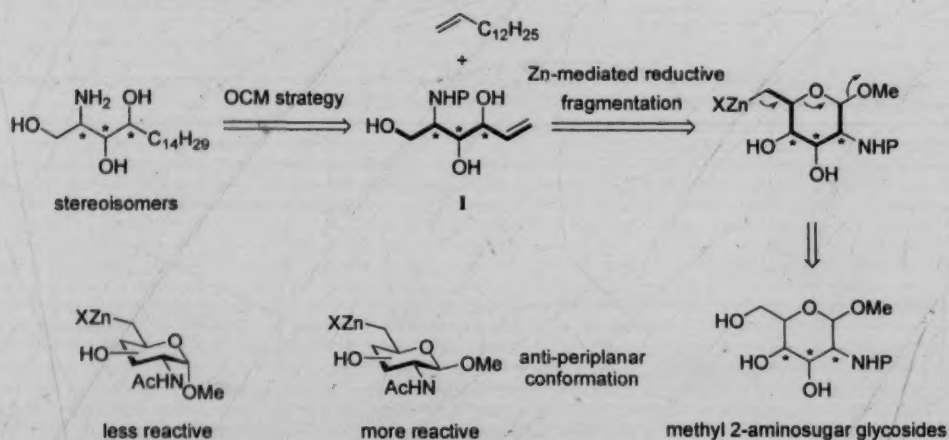


Figure 4.3. Retrosynthetic analysis of C-18-phytosphingosines

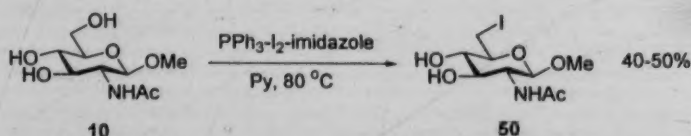
Another limitation of currently published syntheses of sphingolipids (including sphingosine and phytosphingosine) using the OCM strategy is that the synthesis of the truncated precursors are lengthy, and lack of efficiency in spite of their sources from

carbohydrates or diastereoisomer synthesis.^[140,143] Unlike these syntheses, our truncated structures **I** are derived from 2-amino-2-deoxyhexoses because they share the same amino-alcohol constitutional backbone sequence (see dashed line in Figure 4.3.). Actually, D-glucosamine and derivatives have been employed by some groups as the starting materials to prepare phytosphingosine via Wittig olefination as a key reaction.^[142a,144] Unfortunately, none of them is efficient due to long reaction sequences and laborious protection and inversions of configuration.

In our synthetic plan, **I** is envisaged to arise from aldehydes bearing a terminal alkene, which can be readily prepared from 2-amino-2,6-dideoxy-6-halo-hexopyranosides via a reductive fragmentation mediated by zinc powder^[145,146]. In turn, the 6-halogenated glycosides are obtained from their corresponding 2-amino-2-deoxy-hexopyranosides. The advantage of this strategy is that different stereoisomers of phytosphingosines can be conveniently synthesized from the corresponding 2-amino-2-deoxy-hexopyranosides via the same reaction procedure. Due to the antiperiplanar configuration of the carbon-halogen bond and the glycosidic bond of the β -anomer of methyl 2-amino-2-deoxy-hexopyranosides, they are more reactive in the reductive elimination than their α -anomers. In Chapter three, we have already described an expedient and large-scale synthesis of four different 2-acetimo-2-deoxy- β -D-hexopyranosides from inexpensive D-GlcNAc via our furanose oxazoline method. This chemistry will certainly facilitate our proposed C-18-phytosphingosine syntheses.

4.2.2 Synthesis of truncated D-xylo- and D-ribo-C-6-phytosphingosines

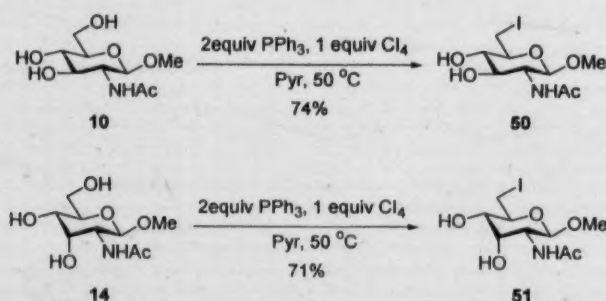
As mentioned above, the synthesis of truncated D-xylo- and D-ribo-C-6-phytosphingosines began with methyl 2-acetamido-2-deoxy- β -D-glucopyranosides **10** and **14**, respectively, which had been prepared over 10 grams from inexpensive D-GlcNAc as described in previous chapters. The Zn-mediated reductive fragmentation of ω -halo glycosides was first developed by Bernet and Vasella in 1978,^[145] and has found broad synthetic application in carbohydrate chemistry. The commonly used halides in this reaction are bromides and iodides. We first examined 2-acetamido-6-bromo-2,6-dideoxy-hexopyranosides as the reaction substrates, but unfortunately it was not successful for fragmentation. Then we turned to choose more reactive 2-acetamido-2,6-dideoxy-6-iodo-hexopyranosides as the substrates. We initially attempted to synthesize these compounds via the conventional iodine-imidazole-triphenylphosphine system at elevated temperatures, which had been previously applied with desirable yields to some monosaccharides.^[147] However, when applied to compound **10**, an undesirable yield (40-50%) with poor selectivity was obtained. Furthermore, the separation of mono- and di-halogenated products proved to be difficult, even using chromatography.



Scheme 4.1 Synthesis of methyl 2-acetamido-2,6-dideoxy-6-iodo- β -D-glucopyranoside **50** using PPh₃-I₂-imidazole

To improve the selective 6-iodination, another system, using triphenylphosphine and carbon tetrahalides in pyridine, was investigated. This system was initially employed

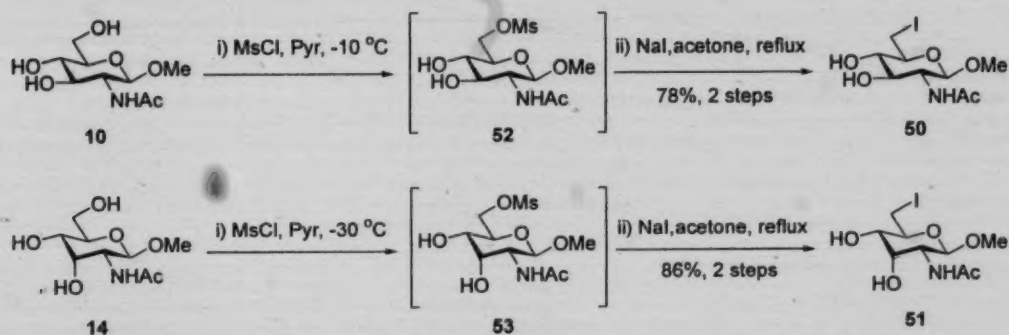
by Whistler *et al.* for the selective halogenation on the 6-OH primary alcohols in mono- and disaccharides. Excellent yields (~98%) have been obtained upon chlorination, bromination and iodination of methyl β -D-glucopyranosides.^[148] The high selectivity was presumably due to the initial formation of a triphenylphosphine dihalide, a bulky halogenating complex, which would favor reaction at the primary alcohol. The treatment of **10** with $\text{PPh}_3\text{-Cl}_4\text{-Pyr}$ was first examined at room temperature but the reaction was found to be sluggish. To accelerate the reaction, the temperature was then elevated to 50 °C and it was found that the reaction proceeded to completion in 5-6 hours, affording methyl 2-acetamido-2,6-dideoxy-6-iodo- β -D-glucopyranoside **50** in 74% yield as a sole product. Treatment of methyl 2-acetamido-2-deoxy- β -D-allopyranoside **14** under the same conditions gave methyl 2-acetamido-2,6-dideoxy-6-iodo- β -D-allopyranoside **51** in 71% yield.



Scheme 4.2. Synthesis of 6-iodo derivatives **50** and **51** using $\text{PPh}_3\text{-Cl}_4\text{-Py}$ system

However, there are limitations to the $\text{PPh}_3\text{-Cl}_4\text{-Py}$ iodination reagent: expensive carbon tetraiodide and separation difficulties when the reaction is scaled up. Therefore, the $\text{PPh}_3\text{-Cl}_4\text{-Pyr}$ method is not well suited to the large-scale synthesis. An alternative approach for large-scale synthesis of methyl 6-iodo glycosides was then developed

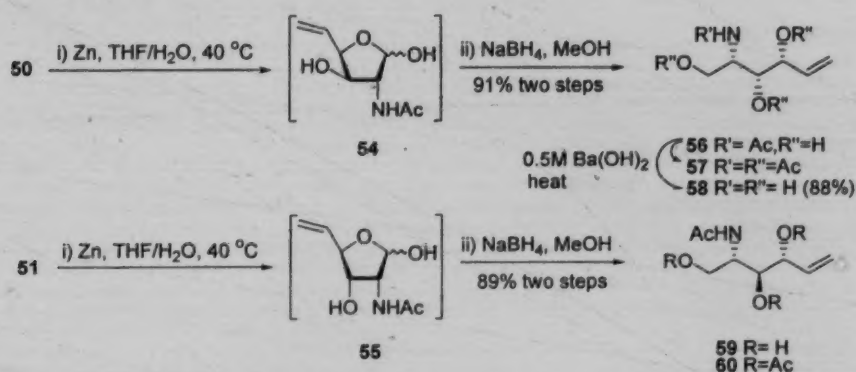
through a two-step procedure involving mesylation, and a displacement reaction with iodide in one-pot procedure (Scheme 4.3). In the first step, selective 6-mesylation of 2-acetamido-2-deoxy-hexopyranosides can be achieved in high yield at a low temperature. For example, the reaction of **10** with 1.1 equivalent of mesyl chloride at -10°C gave the desired 6-mesyated intermediate **52**, which was used directly in the next displacement without chromatography. The 6-*O*-mesyated intermediate **52** was treated with sodium iodide in acetone at reflux to provide compound **50** in a 78% yield over the two steps. Similarly, compound **51** was also rapidly prepared in 86% yield from **14** using the same two-step procedure via the 6-mesyated intermediate **53**. Both synthetic procedures have been successfully scaled to greater than 10-gram quantities.



Scheme 4.3. Synthesis of 6-iodo-derivatives **50** and **51** by a two-step procedure

With compounds **50** and **51** in hand, the ring opening reaction via Zn-mediated reductive fragmentation was then performed as described in Scheme 4.4. This fragmentation is usually carried out in aqueous alcohols at reflux with freshly pre-activated zinc powder. However, the resulting yields are often moderate due to the instability of the aldehyde products and side reactions.^[145,149] Recently, a new protocol involving sonication was introduced by the Madsen group in their fragmentation of

ω -iodo glycosides and excellent yields ($\sim 90\%$) were typically achieved.^[150] This new protocol was therefore applied to our methyl ω -iodo glycoside substrates (Scheme 4.4). Under sonication with freshly pre-activated zinc powder in a mixture of THF and water at 40 °C for 2 hours, compound **50** was completely converted to the desired 2-acetamido-2-deoxy-glycofuranose **54** bearing a terminal double bond, which was clearly characterized by its ¹H-NMR spectrum. After simple filtration, compound **54** was then subjected to NaBH₄ reduction in methanol to give the desired truncated C-6-phytosphingosine **56** with the *D*-xylo configuration in 91% yield over two steps.



Scheme 4.4. Synthesis of truncated *D*-xylo- and *D*-ribo-C-6-phytosphingosines

Likewise, a truncated C-6-phytosphingosine **59** with a natural *D*-ribo configuration was also readily prepared from **51** via **55** by using the same procedure in 89% yield over two steps. Furthermore, the 2-acetamido group of **56** could be smoothly cleaved by treatment with 0.5M Ba(OH)₂ solution at 90 °C to provide the free amino derivative **58** in 88% yield. This two-step procedure, which does not require chromatography, has been successfully scaled to multigram quantities. Compound **56** and

59 were then fully acetylated to give 57 and 60, respectively, for subsequent OCM reactions.

4.2.3 Synthesis of phytosphingosines from truncated structures via OCM

With truncated C-6-phytosphingosines available, their chain elongation to C-18-phytosphingosines was explored by olefin cross metathesis. It is reported that olefin metathesis reactions are commonly conducted in dichloromethane or toluene at reflux in the presence of catalysts. For the synthesis of sphingolipids by OCM chain elongation, an excess of aliphatic alkene (typically 2-5 equiv.) is usually required to suppress homocoupling side reactions. In some cases, microwave heating has been introduced to minimize side reactions to achieve a desirable yield.^[143b, 151] Several kinds of ruthenium catalysts have been employed in OCM chain elongation reactions, including Grubbs' first generation (A) and second generation (B) catalyst as shown in Figure 4.4.^[152] In most of the cases, Grubbs' second generation catalyst has proven to be the best choice because of its high reactivity and insensitivity to water and air. Previous studies have revealed that the successful application of OCM in chain elongation reactions was heavily dependent on the substrates and their protecting groups, the catalyst as well as individual reaction operations.^[138] Therefore, further optimization of the reaction protocols are still needed to obtain satisfactory yields on our C-6-phytosphingosine substrates.

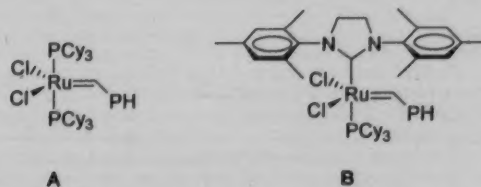


Figure 4.4. Grubbs first (A) and second generation (B) catalysts

Our chain extensions of **57** and **60** by OCM were carried out in dichloromethane at reflux in the presence of Grubbs' second generation (B) catalyst and an excess amount (5 equiv) of 1-tetradecene (Scheme 4.5); the obtained results are summarized in Table 4.1. In most previous reports on OCM, the catalyst was usually added in one portion at the beginning of the reaction. However, we found that the addition protocol of catalyst addition could dramatically affect the reaction outcome. In our initial OCM attempts on **57**, 20% Grubbs second generation catalyst was added in one portion at the beginning of the reactions (protocol A). It was found that more than half of the substrate **57** remained even in the presence of 40% catalyst B or after an extended time (5 days), and the resulting yields of unsaturated *D*-xylo-phytosphingosine tetraacetate **61** were around 30-40% (entry 1-3).

Table 4.1. Studies on olefin cross metathesis of C-6-phytosphingosines with tetradecene

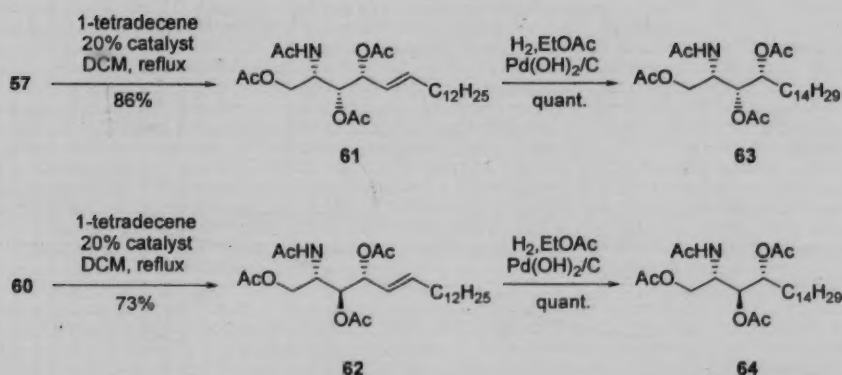
Entry	Substrates	Catalyst B (mol%)	Reaction times	Addition protocol ^b	Product yield (%) ^c
1	57	20	24 h	A	36 ^d
2	57	40	71 h	A	41 ^d
3	57	20	5 days	A	32 ^d
4	57	20	18 h	B	86
5	60	20	18 h	B	73
6	57	10	18 h	B	78

^a All reactions were heated at 40 °C and in the presence of 5 equivalent of 1-tetradecene; ^b Protocol A: catalyst was added in one portion at the beginning of reaction; Protocol B: catalyst was added in 4-5 portions every 2 hours during the reaction; ^c Isolated yields; ^d Starting material (49-55%) was recovered.

However, when 20% catalyst was added evenly in 4-5 portions every 2 hours (protocol B) to substrate **57**, it was surprisingly found that the desired compound **61** was obtained in a remarkably increased yield of 86% (entry 4). The OCM reaction was quite clean and only trace amounts of starting material were observed. Moreover, even if only 10% Grubbs' second generation catalyst was employed to **57** according to protocol B, a good yield with 78% was still observed (entry 6). Protocol B was also successfully applied to substrate **60**, and the desired unsaturated *D-ribo*-phytosphingosine tetraacetate **62** was obtained in 73% yield (entry 5). This dramatic effect of addition protocol of catalyst could be explained by the fact that during the OCM reactions, only a partial amount of the catalyst was involved, and the remaining amount of the catalyst was indeed deactivated.

In all cases, both the resulting unsaturated C-18-phytosphingosine tetraacetates **61** and **62** possess excellent *E/Z* selectivities. The desired *E*-configured double bond at C-5 and C-6 was unambiguously confirmed by a large coupling constant value (>17 Hz) between H-5 and H-6. No corresponding *Z*-isomer was observed based on $^1\text{H-NMR}$ experiments. The *E/Z* selectivity results obtained from our experiment are much better than those reported in related literature ($E/Z = 4:1$).^[143b]

Finally, unsaturated C-18-phytosphingosine tetraacetates **61** and **62** were hydrogenated to the corresponding saturated counterparts **63** and **64** in nearly quantitative yield using $\text{Pd}(\text{OH})_2$ on carbon as the catalyst in ethyl acetate (Scheme 4.5). The spectroscopic and optical data of both **63** and **64** were in full accordance with those reported in the literature,^[137b,142a,h,andj] thereby affording unambiguous proof of their structural and stereochemical integrity.



Scheme 4.5. Synthesis of D-xylo and D-ribo-phytosphingosine tetraacetates **63** and **64**

4.2.4 Conclusions

A concise and flexible synthetic approach to C-18-phytosphingosines from the corresponding 2-acetamido-2-deoxy-hexopyranosides was developed via key reactions of zinc-mediated reductive fragmentation and olefin cross metathesis. Using this approach, two kinds of unsaturated D-xylo and natural D-ribo-phytosphingosine, as well as their saturated counterparts, have been successfully synthesized. This flexible and practical approach will certainly allow us to prepare conveniently a series of phytosphingosine analogues, isomers and derivatives for structure-activity relationship (SAR) studies.

epimer (**69**) (Figure 4.7). We reasoned that the stereo- and enantiomeric outcome of the aldol condensation should be dictated by the (+)-(1*R*,2*R*,5*R*)-2-hydroxy-3-pinanone auxiliary, while the stereochemistry of the aldehydes should have little effect.^[156]

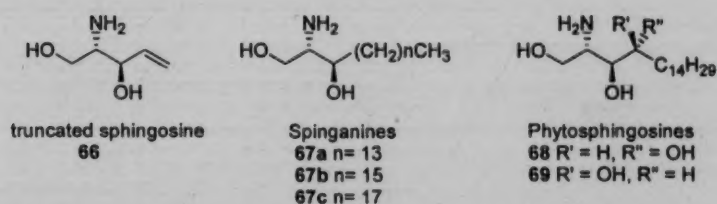
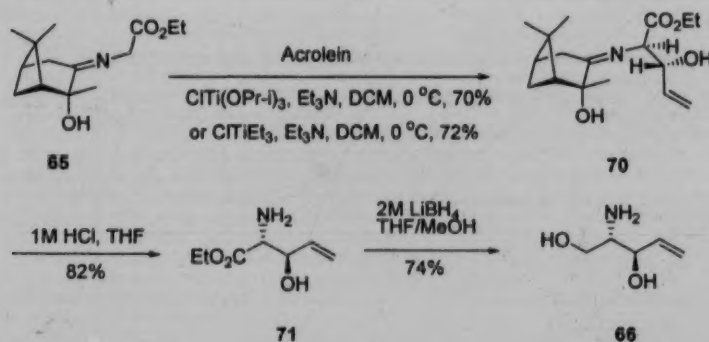


Figure 4.6. The target compounds by asymmetric aldol condensations

4.3.2 Synthesis of truncated sphingosine, spinganines and phytosphingosines

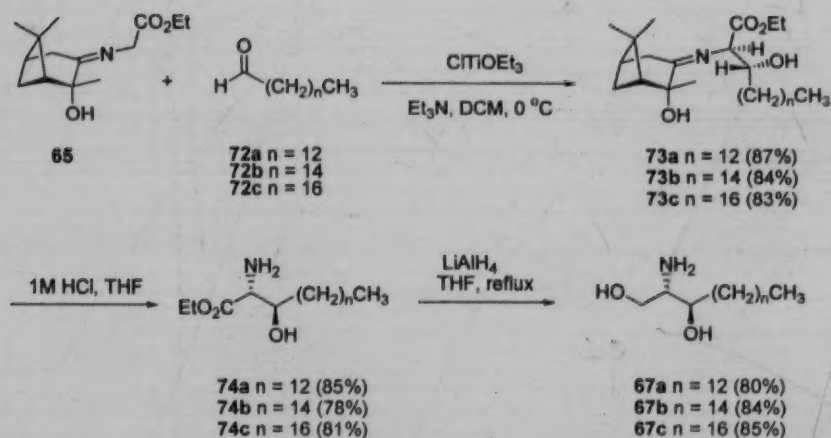
We first examined the asymmetric synthesis of truncated sphingosine **66** by the condensation of an iminoglycinate **65** with acrolein (Scheme 4.6). The iminoglycinate **65** was prepared according to a known procedure by condensing (+)-(1*R*,2*R*,5*R*)-2-hydroxy-3-pinanone with ethyl glycinate.^[157] When the aldol condensation was carried out using $ClTi(OPr-i)_3/Et_3N$ as the reagents, a 1:1 mixture of ethyl and isopropyl esters was obtained, due to partial exchange of the ethoxy group in the glycinate ester with the isopropoxy group of the reagent. This exchange was also observed in Solladié-Cavallo's original publication.^[153] However, the desired adducts **70** ($R_1 = Et$, and *i*-Pr) were obtained in 69% yield and were found to be the only diastereoisomer by ^1H -NMR spectroscopic analysis. To simplify the analysis, we also performed the reaction using $ClTi(OEt)_3/Et_3N$. As expected, the adduct **70** ($R_1 = Et$) was obtained as a single compound in a good yield of 72%, as detected by ^1H -NMR spectroscopy. No 1, 4-Michael addition products were observed in either case. The adduct **70** from both

reactions was smoothly hydrolyzed in 1M HCl in THF to yield **71** in a similar 82% yield. Furthermore, the chiral auxiliary was recovered and could be used to prepare the iminoglycinate **65**. The free amino-esters **71** were reduced with LiBH₄ in a mixture of methanol and THF at room temperature to afford the desired truncated sphingosine analogue **66** in 74% yield.



Scheme 4.6. Asymmetric synthesis of truncated sphingosine **66** using chiral auxiliary **65**

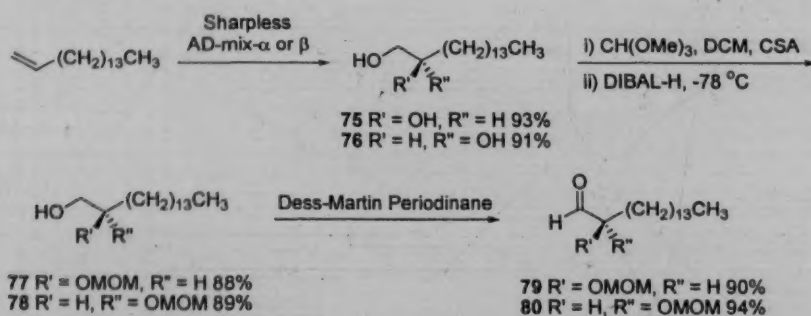
The above strategy was applied to the asymmetric syntheses of sphinganine. In the literature, sphinganine is normally prepared by hydrogenation of expensive sphingosines,^[158] carbohydrates,^[159] or L-serine^[160] in six or more steps. However, using the strategy shown in Scheme 4.7, various sphinganine with different aliphatic chain lengths were successfully prepared in a very concise manner. Treating the iminoglycinate **65** with three aldehydes (**72a-c**) in the presence of ClTi(OEt)₃/Et₃N, the corresponding aldol adducts (**73a-c**) were obtained in yields ranging from 83 to 87%. ¹H-NMR spectra revealed that only one diastereoisomer was obtained in each case. The product imines **73a-c** were hydrolyzed under acidic conditions to afford intermediate amines **74a-c** in high yield (78–85%); once again, the chiral auxiliary was recovered. Final reductions using LiAlH₄ in THF gave the desired sphinganine (**67a-c**) in 80–85%.



Scheme 4.7. Asymmetric synthesis of sphinganines **67a-c** using chiral auxiliary **65**

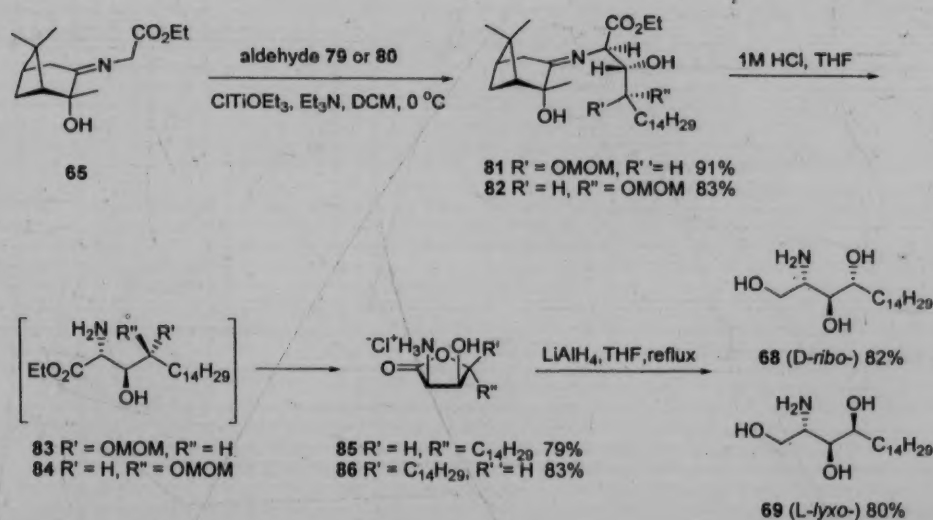
Following the success in the synthesis of truncated sphingosine **66** and sphinganines **67a-c**, the *D-ribo*- and *L-lyxo*-phytosphingosines were the next logical synthetic targets in order to extend this methodology. Phytosphingosines differ from sphingosines and sphinganines by the additional stereocentre at C-4. In order to prepare *D-ribo*-phytosphingosine **68** and its 4-epimer **69**, retrosynthesis revealed that aldehydes **79** and **80** are needed. Both aldehydes could be prepared from a commercially available 1-hexadecene based on a modified procedure as outlined in Scheme 4.8.^[142a] The asymmetric dihydroxylations of 1-hexadecene by the Sharpless AD-mix- α and - β catalysts, respectively, gave the desired compounds **75** and **76** in excellent yield (both over 90%). Then, by treating **75** and **76** with trimethylorthoacetate and a catalytic amount of camphor-10-sulfonic acid followed by *in situ* reduction with DIBAL-H at -77°C , the methoxymethyl group (MOM) was selectively installed at C-2 affording free primary alcohols **77** and **78** in 88% and 89% yields. Their ^1H and ^{13}C NMR spectra are completely consistent with those reported in the literature.^[142a] The final oxidation of **77**

and **78** with Dess-Martin periodinane^[161] gave the desired aldehydes **79** and **80** both in excellent yields (90% and 94%).



Scheme 4.8. Asymmetric synthesis of aldehydes **79** and **80** from 1-hexadecene

With aldehydes **79** and **80** in hand, the condensation with iminoglycine **65** was then conducted (Scheme 4.9). The experiments showed that the condensation proceeded very well, giving **81** and **82** in excellent yields (83-91%) and in high diastereoselectivity. As predicted, the stereochemistry at the α -position of the aldehydes did not influence the stereo- and enantioselectivity of the aldol condensation. Treatment of **81** or **82** with 1M HCl smoothly cleaved both the imine and the MOM protecting group. Intermediates **83** and **84** spontaneously lactonized to afford **85** and **86**, and were isolated as the hydrochloride salt in 79% and 83% yield, respectively. The stereochemistry of the lactones was confirmed by comparing the coupling patterns and the coupling constants between H-2 and H-3 with literature data.^[142a] Finally, both lactones were reduced with LiAlH_4 in THF at reflux to give the desired *D*-ribo-phytosphingosine **68** and its 4-epimer, *L*-lyxo-phytosphingosine **69** in 82% and 80% yield, respectively. The overall yield for the synthesis from 1-hexadecene is greater than 45% in both cases.

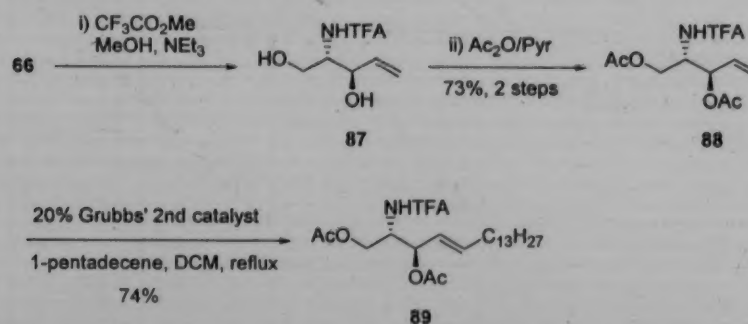


Scheme 4.9. Asymmetric synthesis of D-ribo and L-lyxo-phytosphingosines **68** and **69**

The stereochemistry of the synthesized sphingoids was determined by ¹H-NMR spectroscopy. For example, for compound **66**, in methanol-*d*₄, H-2 appeared as a ddd pattern at 2.76 ppm, with *J*_{1a,2} ~4.5 Hz, *J*_{1b,2} ~6.8 Hz and *J*_{2,3} ~6.4 Hz. H-3 also appeared as a ddd pattern, with *J*_{3,4} ~6.1 Hz. This is in agreement with the coupling patterns of the corresponding protons of the natural sphingosine, as reported in the literature.^[153] It is also consistent with a similar compound prepared by another route.^[139a] For sphinganine derivatives **67a-c**, phytosphingosine **68** and its 4-epimer **69**, unfortunately, most of the literature NMR data were recorded in pyridine-*d*₃, DMSO-*d*₆ or chloroform-*d*. In these solvents, due to complications resulting from coupling with the attached hydroxyl protons as well as aggregation in some solvents (such as chloroform-*d*) most of the key protons appear either as broad resonances or as multiplets. Rarely were coupling constants for these protons reported. We discovered all these compounds gave well-resolved NMR spectra in methanol-*d*₄. With the suppression of coupling to the hydroxyl protons, most of the key coupling constants could be extracted. For example, for

sphinganine **67b**, H-2 resonated at 2.69 ppm as a ddd and H-3 at 3.48 as a ddd, with $J_{1a,2} \sim 4.2$ Hz, $J_{1b,2} \sim 7.6$ Hz and $J_{2,3} \sim 5.4$ Hz. For phytosphingosine **68**, H-2 appeared as a ddd at 2.94 ppm, H-3 as a dd at 3.33 ppm and H-4 as a ddd at 3.51 ppm, with $J_{1a,2} \sim 4.2$ Hz, $J_{1b,2} \sim 6.6$ Hz, $J_{2,3} \sim 5.7$ Hz and $J_{3,4} \sim 7.8$ Hz. In addition, for the phytosphingosine 4-epimer **69**, H-2 appeared as a ddd at 2.93 ppm, H-3 as a dd at 3.32 ppm and H-4 as a ddd at 3.66 ppm, with $J_{1a,2} \sim 4.2$ Hz, $J_{1b,2} \sim 7.0$ Hz, $J_{2,3} \sim 7.0$ Hz and $J_{3,4} \sim 2.7$ Hz. That the compounds have the same (2*S*,3*R*)-D-*erythro* configuration is supported by the observation that similar $J_{2,3}$ coupling constants (5.4–7.0 Hz) were observed for the newly formed C2–C3 linkages when compared to **65** ($J_{2,3} \sim 6.4$ Hz). Optical rotations were also consistent with literature reports.^[142a,162,163]

4.3.3 Synthesis of C-18-sphingosine derivative via olefin cross metathesis



Scheme 4.10. Synthesis of C-18-sphingosine derivative **89** via OCM strategy

The truncated sphingosine **66**, which was readily synthesized above from acrolein, was also successfully extended to a 4(*E*)-C-18-sphingosine derivative **89** by olefin cross metathesis (Scheme 4.10). Although there are many choices available for 2-amino protection, we decided to select the trifluoroacetyl group because it had not yet been

employed in the OCM reaction, and can be easily cleaved together with other ester groups, thus facilitating our subsequent synthesis of GM₃ and GM₂ gangliosides.

The treatment of **61** with methyl trifluoroacetate in methanol in the presence of triethylamine afforded a TFA protected intermediate **87**, which was subsequently acetylated by standard acetylation conditions to give the desired compound **88** in 73% yield over two steps. Then, by reaction with 1-pentadecene using 20% Grubbs second generation catalyst according to protocol B of Table 4.1, compound **88** was successfully extended to the desired sphingosine **89** with complete *E*-stereoselectivity in a 74% yield. The *E*-configuration of **89** was characterized by a ¹H-NMR experiment, and a large *J*_{4,5} coupling constant 15.2 Hz clearly revealed the presence of an *E*-configured double bond. The successful elongation of **88** by the OCM reaction validates our initial concept of a truncated sphingosine as a valuable tool in the design and synthesis of a series of *E*-sphingosine and analogues.

4.3.4 Conclusions

We have demonstrated that the (2*S*,3*R*)-*D*-*erythro*-amino alcohol chiral element, commonly found in a wide range of sphingoid bases, can be synthesized in very good yields with high diastereoselectivity by an aldol condensation between a chiral auxiliary and the appropriate aldehydes. The chain extension of a truncated sphingosine structure by the OCM strategy was also successfully demonstrated. This methodology has been shown to be practical, highly versatile, and amenable to the synthesis of structurally elaborate compounds related to sphingolipids.

Chapter Five

Synthesis of α -Galactosylceramides and β -Lactosylceramides via Olefin Cross Metathesis

5.1 Introduction

The discovery of α -galactosylceramide (α GalCer) as powerful immunostimulant of natural killer T (NKT) cells and potent antitumor agent has promoted an increasing demand for this compound, and its analogues, for further biological investigations.^[25] Owing to their limited availability from natural resources, the chemical synthesis of α -galactosylceramides and analogues is essential, and several syntheses have been developed in recent years. In comparison with most other GSLs, the synthesis of α -galactosylceramides is quite difficult due to the formidable stereoselective formation of the α -linkage between glycosyl donors and a bulky acceptor ceramide or phytosphingosine.

α -Galactosylceramides and several analogues were first synthesized by the Kirin Brewery company using a 2,3,4,6-tetrabenzyl galactosyl fluoride donor and the corresponding ceramide acceptors.^[21,164] Since then, several other types of galactosyl donors such as bromides,^[165] trichloroacetimidates^[166] and thioglycosides^[166b] have also been explored for α -linkage formation of galactosylceramides. However, glycosylations involving these galactosyl donors often suffered from relatively low yields and sometimes poor α/β anomeric selectivity. For example, the yield of galactosyl fluoride^[21, 39,164,165b, 167] typically range between 30 to 60%, and are complicated by the formation of the β -anomer, which is hard to separate. Although the anomeric selectivity can be

improved by the use of galactosyl trichloroacetimidates, the reaction usually requires portionwise addition of both the donor and acceptor, otherwise yields drop markedly. In the case of thioglycosides and galactosyl bromides, both yields and anomeric selectivity are compromised. Recently, a galactosyl iodide was utilized by Gervay-Hague as the donor to construct α -linked glycosides and both high yield and anomeric selectivity could be obtained for α -galactosylceramide by using this kind of donor.^[168] Besides the exploration of different donors, another effective strategy was also introduced by Kiso's group particularly for the synthesis of α -galactosides. In their strategy, a very bulky di-*tert*-butylsilyl (DTBS) group was installed at the C-4,6 position of a *galacto*-configuration sugar to direct a highly α -selective glycosylation, in spite of the presence of a participating group at the C-2 position such as benzoyl or Troc group. The desired α -galactosylceramide has been successfully prepared in around 60% yield through this strategy.^[169]

Although some progress has been achieved so far, the efficient synthesis of α -galactosylceramide still remains a major challenge for carbohydrate chemists. To address this challenge, we have developed herein a general and flexible approach to effectively prepare α -galactosylceramide and analogues employing the olefin cross metathesis (OCM) strategy. Despite its recent applications in the synthesis of β -galactosylsphingosine,^[170] to the best of our knowledge, the OCM strategy has not yet been exploited in the more challenging α -galactosylceramide synthesis.

5.2 Retrosynthetic analysis

Our retrosynthetic analysis of α -galactosylceramide is illustrated in Figure 5.1 and two general synthetic routes (routes A and B) are proposed. Both routes employ a galactosyl donor with a non-participating group at C-2 position such as a benzyl group to construct the desired α -linkage. In route A, a commonly used synthetic strategy for most glycolipids, α -galactosylceramide **90** is disconnected to a 2,3,4,6-tetrabenzyl galactosyl donor **II** and a large acceptor-ceramide **III**, which can be prepared from the *N*-acylation of C-18-phytosphingosine **V** with a corresponding fatty acid. The C-18-phytosphingosine **V** is then derived from a 3,4-protected truncated C-6-phytosphingosine **VII** via an OCM reaction with 1-tetradecene as described in Chapter four. By using OCM reactions, a series of phytosphingosine analogues and derivatives with different chain lengths or functional groups can be easily prepared and then incorporated into α -galactosylceramide for structural modifications or other purposes.

Although route A is quite straightforward from a synthetic viewpoint, the glycosylation is not satisfactory in terms of the yield and anomeric selectivity due to the use of a bulky ceramide as the acceptor. To circumvent this drawback in route A, an alternative route B was proposed in our synthesis of α -galactosylceramide and analogues. In route B, the target compound **90** is prepared from α -galactosylphytosphingosine **IV**, which is envisaged from compound **VI** bearing a truncated C-6-phytosphingosine moiety and 1-tetradecene via an OCM reaction. The compound **VI** can be obtained from the glycosylation of a truncated C-6-phytosphingosine **VII** with a suitable galactosyl donor **II**. Since a much smaller aglycon **VII** is employed as the acceptor, an improved yield and anomeric selectivity can be anticipated from route B. Like route A, various lipid

chains and functional groups can also be conveniently introduced to the truncated C-6-phytosphingosine moiety via OCM reactions in route B. Moreover, compared with route A, route B is more flexible and efficient for constructing a series of α -galactosylceramide analogues and derivatives when considering **VI** as a common building block.

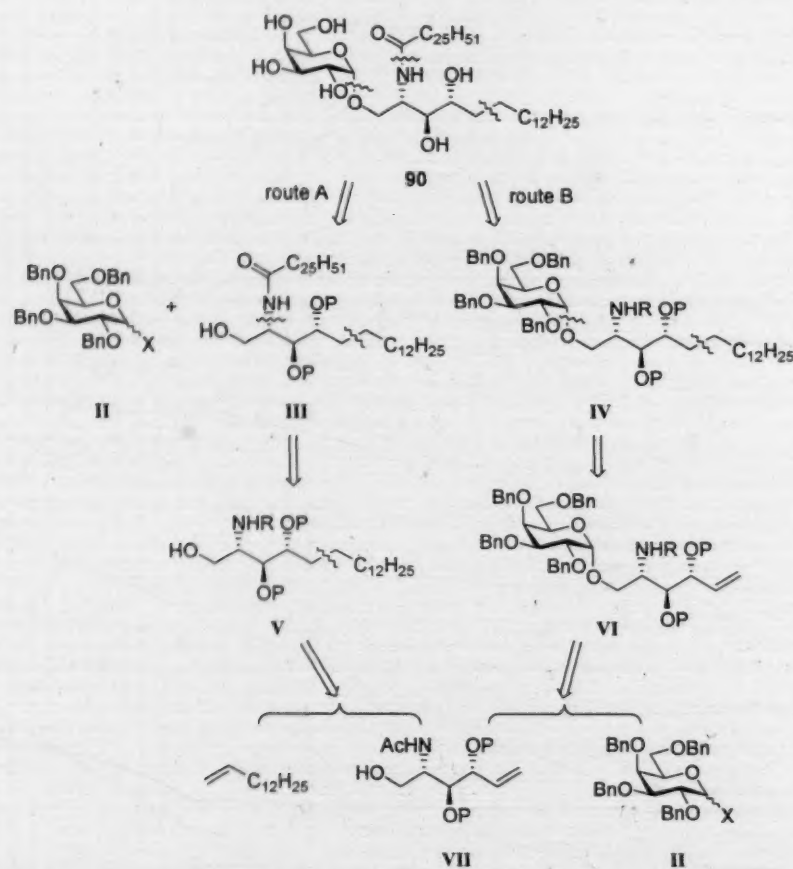
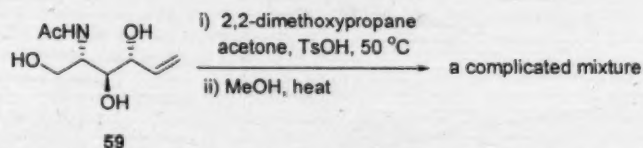


Figure 5.1. Retrosynthetic analysis of α -galactosylceramide (KRN7000) **90**

5.3 Synthesis of 3,4-protected truncated C-6- and C-18-phytosphingosines

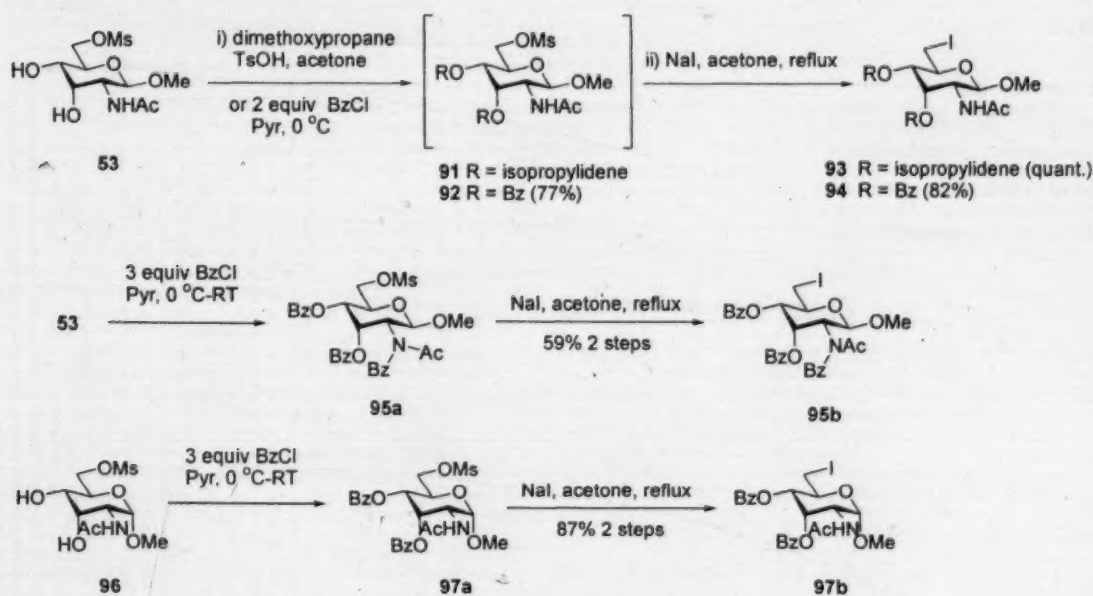
As illustrated in the retrosynthetic plan, both synthetic routes start from a 3,4-protected truncated structure VII. This truncated structure VII could be derived from compound **59**, which has been rapidly prepared from the corresponding 2-acetamido-2-deoxy- β -D-hexopyranoside **14** as described in Chapter four. In this context, an isopropylidene acetal group was first chosen as the protecting group to block selectively the 3,4-vinical alcohol. A direct and selective installation of an isopropylidene group at the 3,4-diol of phytosphingosines has previously been achieved by our group,^[176] and other groups,^[165b] in over 70% yields. The reaction was carried out with 2,2-dimethoxypropane in dry acetone with a catalytic amount of *p*-toluenesulfonic acid and then heated with methanol for 1 hour. However, when applying the same condition to substrate **59** with the *D-ribo* configuration, we unfortunately observed a complicated mixture, which was difficult to separate by chromatography on silica gel (Scheme 5.1).



Scheme 5.1. Our initial attempt at 3,4-selective protection of **59** by isopropylidene acetal

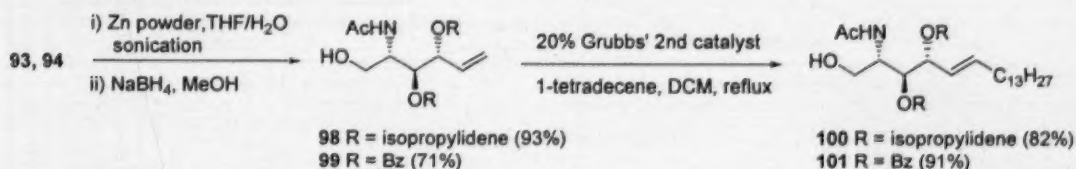
Next we turned our efforts to introduce the protecting groups at an earlier stage (Scheme 5.2). We found that an isopropylidene acetal could be easily installed at the C-3 and C-4 position of D-allosamine derivatives **53** because of its *cis*-3,4-diol configuration, by treatment with 2,2-dimethoxypropane in dry acetone in the presence of a catalytic amount of *p*-toluenesulfonic acid. After simple extraction and without chromatography, the resulting crude residue **91** was then subjected to displacement by reaction with

sodium iodide in acetone at reflux, affording the desired methyl 2-acetamido-2,6-dideoxy-6-iodo-3,4-*O*-isopropylidene- β -D-allopyranoside **93** in almost quantitative yield. Besides 3,4-isopropylidene acetal protection, electron-withdrawing benzoyl groups were also employed to protect the 3,4-diol of **53** in 77% yield by reaction with 2 equiv benzoyl chloride in pyridine at 0 °C. The resulting 6-mesylate compound **92** was then converted to the corresponding 6-iodo-derivative **94** in 82% yield by displacement with sodium iodide in acetone at reflux. It was noted that the treatment of **53** with an excess amount of benzoyl chloride (e.g. over 3 equiv) would rapidly give a tribenzoyl derivative **95a**, while the treatment of its α -counterpart **96** by same conditions (3 equiv benzoyl chloride) generated a dibenzoyl derivative **97a** (Scheme 5.2). Both **95a** and **97a** were converted to their corresponding 6-iodo derivative **95b** and **97b**, respectively. This phenomenon was likely caused by the difference in steric hindrance at C-2 for α - and β -anomers.



Scheme 5.2. The studies on 3,4-protection of **53** and its α -counterpart **96**

With 6-iodo-derivatives **93** and **94** in hand, a two-step procedure of reductive fragmentation and NaBH₄ reduction as described in Chapter four was followed (Scheme 5.3). It was found that **93** was smoothly converted to the desired 3,4-isopropylidene-protected truncated C-6-phytosphingosines **98** in excellent yield (93%) and the synthesis could be easily scaled up to multi-gram quantities. Likewise, by treatment with same procedure, 3,4-di-*O*-benzoylated compound **94** was transformed to the corresponding truncated C-6-phytosphingosine **99** in 71% yields. Next, both **98** and **99** were extended to their C-18-phytosphingosine derivatives via OCM reactions. The OCM reactions with 1-tetradecene were carried out following protocol B as described in Table 4.1. Two desired unsaturated C-18-phytosphingosine derivatives **100** and **101** were obtained, respectively, in 82% yield and 91% yield with excellent *E/Z* stereoselectivity (*E/Z* = ~16:1 for **100**, ~14:1 for **101**). The *E*-configured double bond of **100** and **101** was determined by the large ³*J*_{5,6} coupling constant (15.3 Hz).

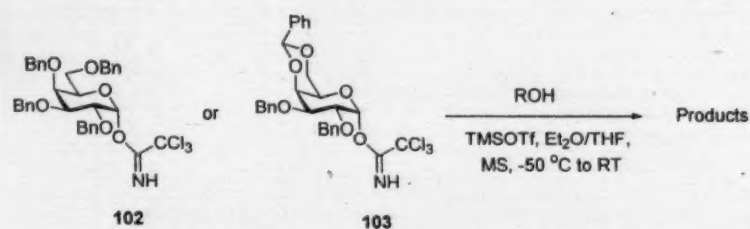


Scheme 5.3. Synthesis of 3,4-protected truncated C-6 phytosphingosines and unsaturated phytosphingosines

5.4 Synthetic efforts toward α -galactosylation using galactosyl imidate donors

With the 3,4-protected truncated C-6-phytosphingosines and unsaturated C-18-phytosphingosine available, the elaboration of α -glycosylceramides was then investigated. The first donor we have examined was galactosyl trichloroacetimidates **102**

and **103** due to their accessibility and common use in oligosaccharide synthesis. All glycosylations were carried out under the promotion of trimethylsilyl trifluoromethanesulfonate in a mixture of Et₂O and THF (10:1, v/v) at a temperature from -50 °C to RT; the results are summarized in Table 5.1. When 2,3,4,6-tetra-*O*-benzylgalactosyl trichloroacetimidate **102** was employed as the donor, in the case of the isopropylidene acetal protected acceptors **98** and **99** (entry 1 and 2), good to high yields (~80%) but poor α/β stereoselectivities were obtained (To avoid difficult separations, the presence and ratios of β -anomers were determined by ¹H-NMR spectroscopy). Improved anomeric selectivity could be obtained from the acceptors with electron-withdrawing protecting groups. For example, a high yield (82%) and increased anomeric selectivity ($\alpha/\beta = 6:1$) was observed from acceptor **99**, and the desired α -product **108** was isolated from its β -anomer by silica gel chromatography (entry 3). This observation is in agreement with previous results reporting the effect of armed and disarmed protecting groups on α -galactosylation.^[166c] On the other hand, the benefits of using 4,6-*O*-benzylidene protected trichloroacetimidate donor **103** on α -galactosylation are known.^[166a,b] There are examples that show in the galactose series the *cis*-decalin ring system with an equatorial phenyl group hinders the attack of the acceptor from β -face.^[171] However, there is no improvement in anomeric selectivity in our case when **103** was employed as the donor (entry 5).

Table 5.1. Glycosidation studies using trichloroacetimidate donors **102** and **103**

Entry	Glycosyl donors ^a	Glycosyl acceptors	Yields (%) ^b	Anomeric selectivity (α/β) ^c
1	101	98	83	1:1
2	101	98	76	3:2
3	101	99	82	6:1
4	101	101	69	2:1
5	103	100	63	1:1

^a The ratio of donors to acceptors (1.5:1); ^b Isolated yields; ^c α/β ratio was judged by ¹H-NMR

5.5 Synthetic studies of α -galactosylation using a galactosyl iodide as the donor

The unsatisfactory α/β anomeric selectivity observed for galactosyl trichloroacetimidate donors prompted us to investigate further other suitable glycosyl donors. The halide ion method, which was first introduced by Lemieux,^[172] has been extensively utilized to elaborate 1,2-*cis*- α -linked glycosides. This method employs a glycosyl bromide as the donor and tetraethylammonium bromide as the promoter. The reaction presumably proceeds via an *in situ* anomerization of the α -glycosyl bromide to a more reactive β -anomer, which subsequently undergoes a S_N2-like displacement to give predominantly the desired α -glycosides. However, the reaction with glycosyl bromide donors is slow and usually several days are needed to complete the glycosylation reactions.^[173] Recently, Gervay-Hague *et al.* reported that α -galactosyl iodide **104** would undergo the *in situ* anomerization and subsequent displacement orders of magnitude

faster than the corresponding glycosyl bromide under the promotion of TBAI (Figure 5.2). By employing a glycosyl iodide donor, they have synthesized many α -glycosides including α -galactosylceramides in good yields and excellent stereoselectivity.^[168,174] Therefore, we decided to utilize this galactosyl iodide as the donor in our subsequent glycosylation for the elaboration of α -galactosylceramides.

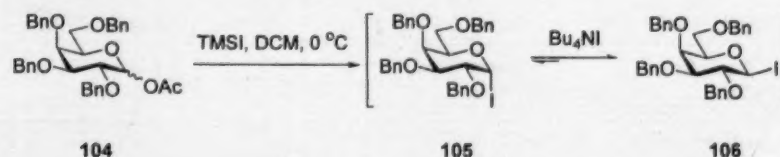
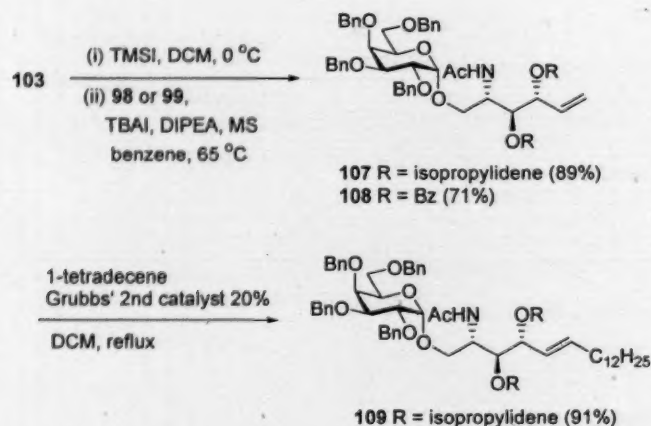


Figure 5.2. *In situ* formation and anomerization of α -glycosyl iodide **105**

The galactosyl iodide donor **105** was generated *in situ* from the 2,3,4,6-tetra-*O*-benzyl-galactosyl acetate **104** according to a known procedure^[174a] as described in Figure 5.2. The treatment of **104** with trimethylsilyl iodide in dry dichloromethane at 0 °C gave α -galactosyl iodide **105**, which would rapidly anomerized *in situ* to its more reactive β -anomer counterpart **107** in the presence of TBAI under heating conditions. The glycosidation of **105** with a truncated structure **98** was then immediately carried out under the promotion of TBAI in the presence of DIPEA and 4Å molecular sieves in benzene at 65 °C to afford exclusively the desired α -glycoside **107** in a high yield (89%) (Scheme 5.4). Similarly, by using the same conditions, the benzoyl protected α -glycoside **108** was also obtained from glycosidation of **102** with acceptor **99** in 71% yield with complete α -selectivity. In these glycosidations, no corresponding β -anomer was observed. The low $J_{1,2}$ coupling constant value (3.8 Hz) observed from ^1H -NMR spectra of **107** and **108** unambiguously established the α -anomeric configuration. Having **107** in

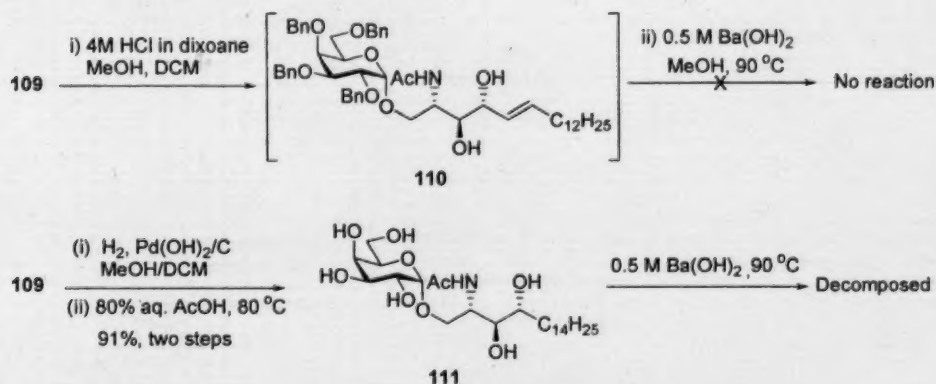
hand, its chain elongation was next performed by an OCM reaction. Using 20% Grubbs' second generation catalyst and following protocol B in Table 4.1, compound **104** was successfully extended to the desired α -galactoside **109** bearing a C-18-phytosphingosine moiety in 91% yield with complete *E*-selectivity. The trans double bond of C-18-phytosphingosine moiety of **109** was ascertained by observation of a large $^3J_{5,6}$ coupling constant (15.3 Hz).



Scheme 5.4. Synthesis of α -galactosylphytosphingosine **109**

Next, the removal of the *N*-acetyl group of **109** proved to be extremely difficult. As shown in Scheme 5.5, compound **109** was first treated with 4M HCl in dioxane (100 μ l) in a mixture of dichloromethane/methanol (5 ml) to remove the isopropylidene acetal, affording **110** before being subjected to the de-*N*-acetylation condition. In Chapter four, we had succeeded in de-*N*-acetylation of a truncated C-6-phytosphingosine **56** in a high yield (88%) by heating with 0.5M Ba(OH)₂. However, when this condition was applied to **110**, it was found that de-*N*-acetylation did not proceed after prolonged reaction times (2 days); most of starting material was recovered. Considering the poor solubility of **110** in

the mixture of 0.5M Ba(OH)₂ and methanol, we decided to remove benzyl groups and isopropylidene acetal of **109** to improve solubility in aq. Ba(OH)₂ solution. The cleavage of the benzyl groups and isopropylidene acetal of **109** was carried out by standard hydrogenolysis over Pd(OH)₂ catalyst on carbon followed by treatment with 80% aqueous acetic acid at 80 °C to yield a quite polar compound **110** in 91% yield for two steps. Unfortunately, compound **111** decomposed after heating with 0.5M Ba(OH)₂ at 90 °C overnight, although it was soluble in aqueous solution.

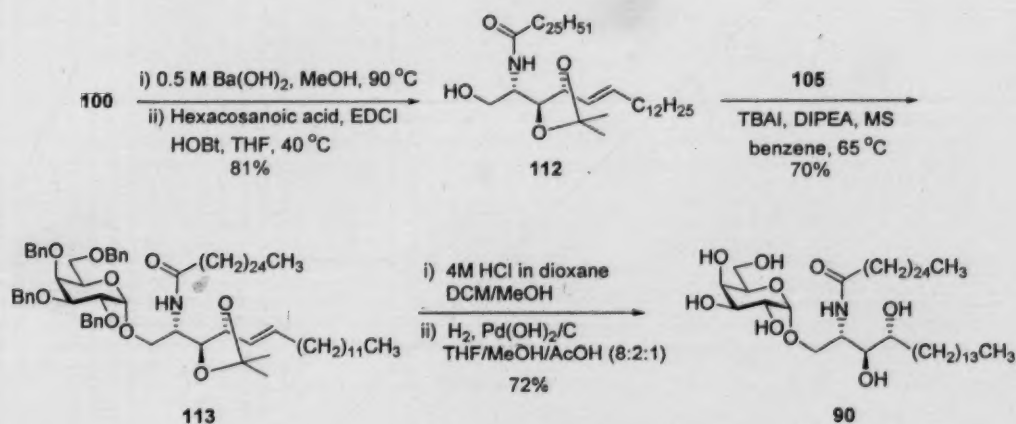


Scheme 5.5. De-*N*-acetylation studies of α -galactosylphytosphingosine **109**

5.6 Elaboration of α -galactosylceramide (KRN7000) via route A

The failure in de-*N*-acetylation of α -galactosylphytosphingosine **109** prompted us to return to route A for the elaboration of α -galactosylceramide (Scheme 5.6), in which the de-*N*-acetylation takes place before α -glycosylation to avoid the decomposition during the harsh de-*N*-acetylation conditions we have to use. In route A, when treated with 0.5M Ba(OH)₂ in methanol at 90 °C for 18 h, the 2-*N*-acetyl group of C-18-phytosphingosine **100** was found to be successfully removed. Then, subsequent

N-acylation with hexacosanoic acid in the presence of EDCI and HOBt in THF at 40 °C gave the desired ceramide **112** in 81% yield over two steps. Next, the stereoselective glycosylation was carried out by reaction of galactosyl iodine **105** with ceramide **112** under the same condition as we described for **107** to provide the desired α -glycoside **113** as a sole product in 70% yield. After the removal of isopropylidene acetal by heating with 4M HCl in dioxane at room temperature and followed by a standard hydrogenation using 20% Pd(OH)₂/C in ethyl acetate, the target product α -galactosylceramide (KRN7000) **90** was successfully obtained in 72% over two steps.



Scheme 5.6. Synthesis of α -galactosylceramide (KRN7000) **90** via route A

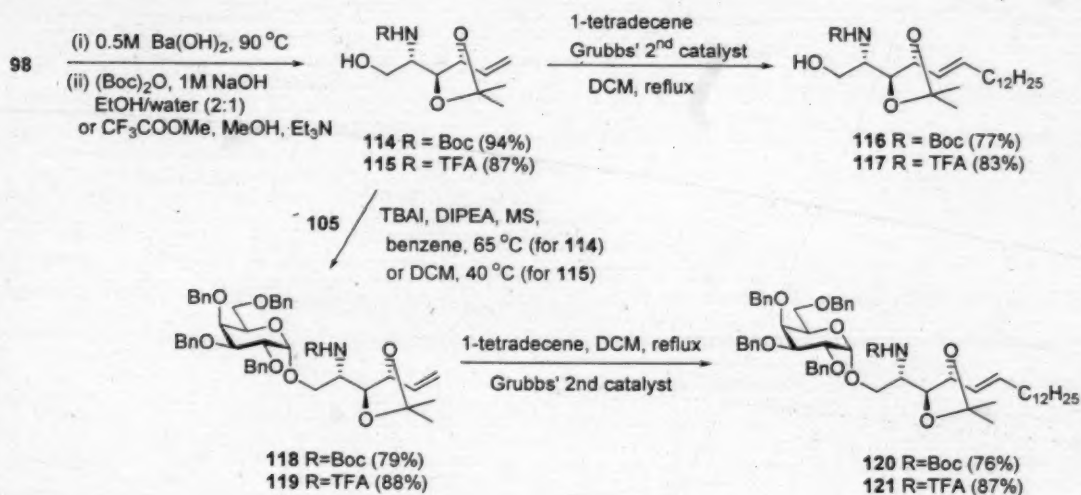
5.7 Elaboration of α -galactosylceramide (KRN7000) via synthetic route B

Following the successful synthesis of α -galactosylceramide (KRN7000) via route A, the more flexible route B was then re-investigated. To avoid the difficulty of *N*-deacetylation that occurred for compound **109**, *N*-acetyl group of **98** was replaced by an acidic labile Boc group and a base labile TFA groups respectively before α -glycosylations (Scheme 5.7). By treatment with 0.5M Ba(OH)₂ at 90 °C, the *N*-acetyl

group of **98** was smoothly removed to give a free amino intermediate, which was then readily protected by reaction with (Boc)₂O in ethanol and water (2:1) in the presence of 1 M NaOH to afford the desired compound **114** in excellent yield (94%). Alternatively, reaction with methyl trifluoroacetate in methanol in the presence of Et₃N provided **115** in 87% yield. Both truncated C-6-phytosphingosines **114** and **115** could be readily extended to the corresponding C-18-phytosphingosines **116** in 77% yield and **117** in 83% yield respectively with excellent *E*-stereoselectivities (*E/Z* = ~ 12:1 for **116**, only *E*-isomer was observed in ¹H-NMR for **117**), by reaction with 1-tetradecene in dichloromethane at reflux using 20% Grubbs' second generation catalyst following the protocol B described in Table 4.1.

With compounds **114** and **115** in hand, their α -glycosylation by the galactosyl iodide donor **105** were then followed according to route B (Scheme 5.7). The glycosylation of **114** with **105** was carried out under the same conditions as described above to afford the desired α -glycoside **118** (79%) with high anomeric selectivity (α/β = ~10:1, judged by ¹H-NMR spectroscopy). However, the glycosylation of **115** with **105** was conducted in dichloromethane at reflux instead of heating at 65 °C in benzene, because it was found that **115** with a polar TFA group was not soluble in hot benzene. The desired product **119** was thus obtained in a high yield (88%) and with exclusive α -anomeric selectivity. The α -anomeric configuration of both **118** and **119** was confirmed by a small *J*_{1,2} value (3.8 Hz). Next the chain elongations of **118** and **119** were performed by OCM reactions with 1-tetradecene using 20% Grubbs second generation catalyst. Thus, following protocol B in Table 4.1, two target compounds, **120** and **121**, both bearing a C-18-phytosphingosine moiety, were formed in 76% yield and 87% yield,

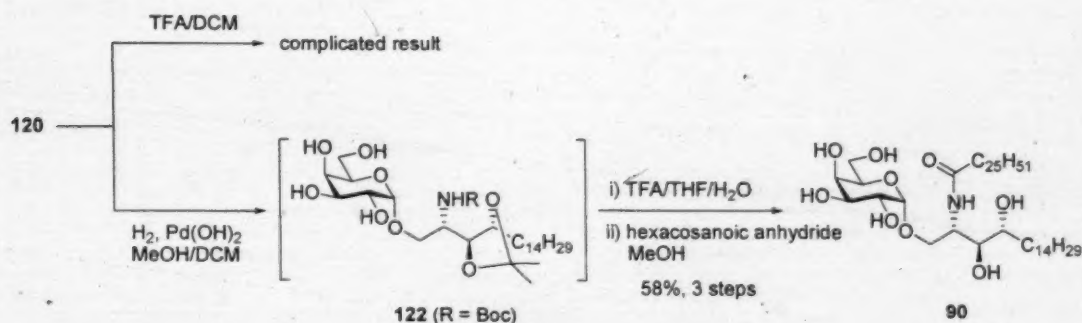
respectively, and with excellent *E*-stereoselectivities (trace amount of isomer for **120**, *E*-isomer only for **121**). The presence of the *trans* double bond in **120** and **121** was clearly indicated by a large *trans* coupling constant ($J_{5,6} = 15.3$ Hz).



Scheme 5.7. Synthesis of C-18-phytosphingosines **116** and **117** as well as α -galactosylphytosphingosines **120** and **121**

In the final elaboration stage of α -galactosylceramide **90**, both the Boc group of **120** and the TFA group of **121** had to be cleaved to allow *N*-acylation with stearic acid. In the case of **120**, we initially planned to remove the Boc group and isopropylidene acetal simultaneously by treating with a common deprotection conditions of 50% trifluoroacetic acid in dichloromethane.^[175] However, a complicated mixture resulted was difficult to separate. Furthermore, another common condition of 4M HCl in dioxane was also examined but the deprotection reaction was still not clean. The complicated mixture was presumably due to the partial cleavage of benzyl protecting group during acidic treatment. Therefore, to avoid any complications caused by benzyl group removal, a

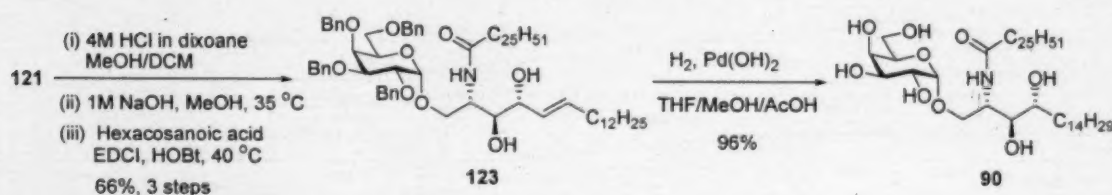
standard hydrogenation was applied to **120** before the acidic removal of the Boc group (Scheme 5.8). The hydrogenolysis of **120** was conducted in the presence of 20% Pd(OH)₂/C catalyst in dichloromethane and methanol to give the debenzylated compound **122** quantitatively. Without purification, compound **122** was immediately subjected to acid treatment with 50% trifluoroacetic acid in THF followed by *N*-acylation with hexacosanoic anhydride in methanol to successfully furnish the final product α -galactosylceramide (KRN7000) **90** in 58% yield over three steps.



Scheme 5.8. Synthesis of α -galactosylceramide (KRN7000) **90** from **120**

Meanwhile, we also investigated another convergent route to α -galactosylceramide **90** starting from **121** as depicted in Scheme 5.9. This route has been shown to be less troublesome compared to the above route from **120**. First, the isopropylidene acetal of **121** was cleanly cleaved by 4M HCl in dioxane and then the removal of TFA group was done by treatment with 1M NaOH in methanol, affording the amino compound. The resulting amino compound was then immediately condensed with hexacosanoic acid in the presence of EDCI and HOBt in dichloromethane at 40 °C to provide α -galactosylceramide derivative **123** in 66% yield over 3 steps. The final hydrogenolysis of **123** with 20% Pd(OH)₂ catalyst on carbon in a mixture of

THF/MeOH/AcOH (8:2:0.5) readily afforded the target product **90** in 96% yield. Unlike most literature NMR data, which are recorded in pyridine-*d*₅, our NMR experiments for **90** were measured in a mixture of CDCl₃ and CD₃OH (5:1, v/v) but both ¹H- and ¹³C-NMR spectra are in complete agreement with those previously obtained by another route in our group.^[176] The observed optical activity [α]_D +42.3 (c 0.35, pyridine) of **90** was also consistent with those reported in the literature [α]_D +43.6 (c 1.0, pyridine).^[164]



Scheme 5.9. Synthesis of α -galactosylceramide (KRN7000) **90** from **121** via route B

5.8 Synthesis of β -lactosylceramide via olefin cross metathesis

Encouraged by its successful application in the synthesis of α -galactosylceramide (KRN7000), the olefin cross metathesis strategy was also exploited in the synthesis of β -lactosylceramide **124** and further extended to the efficient and flexible syntheses of GM₃ and GM₂ gangliosides by combining with enzymatic methods.

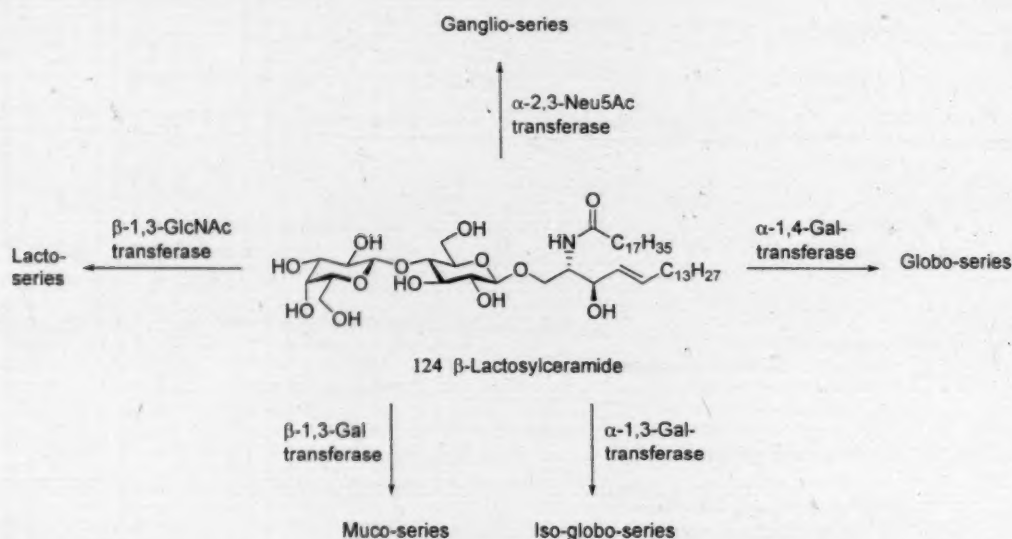


Figure 5.3. The glycolipid series derived from β -lactosylceramide **124**

It is well known that β -lactosylceramide **124** is the most common biosynthetic precursor of GSLs series found in higher animals. As shown in Figure 5.3, many important GSLs such as ganglio-, lacto-, globo- muco- and iso-globo-series are all derived from β -lactosylceramide as a common precursor catalyzed by a variety of suitable glycosyltransferases.^[177] Therefore, from a synthetic point of view, a general and flexible synthetic approach to β -lactosylceramide is essential for the construction of GSLs and analogues. Most syntheses of β -lactosylceramide are usually reported as

integrated parts of syntheses of more complex GSLs.^[178] To our knowledge, so far the OCM strategy mainly focused on the synthesis of monosaccharide-containing GSLs and has not been applied to syntheses of di- or higher saccharide-containing GSLs including lactosylceramide and gangliosides.

Our synthesis of β -lactosylceramide **124** began with a truncated azido-sphingosine **126***, which has been efficiently prepared from 1,2-*O*-isopropylidene-glucofuranose **125** on a 50-100 gram scale in our group.^[139a] Recently, another truncated sphingosine compound **127** was also efficiently synthesized by our group from commercially available D-arabitol in 5 steps (Figure 5.4).

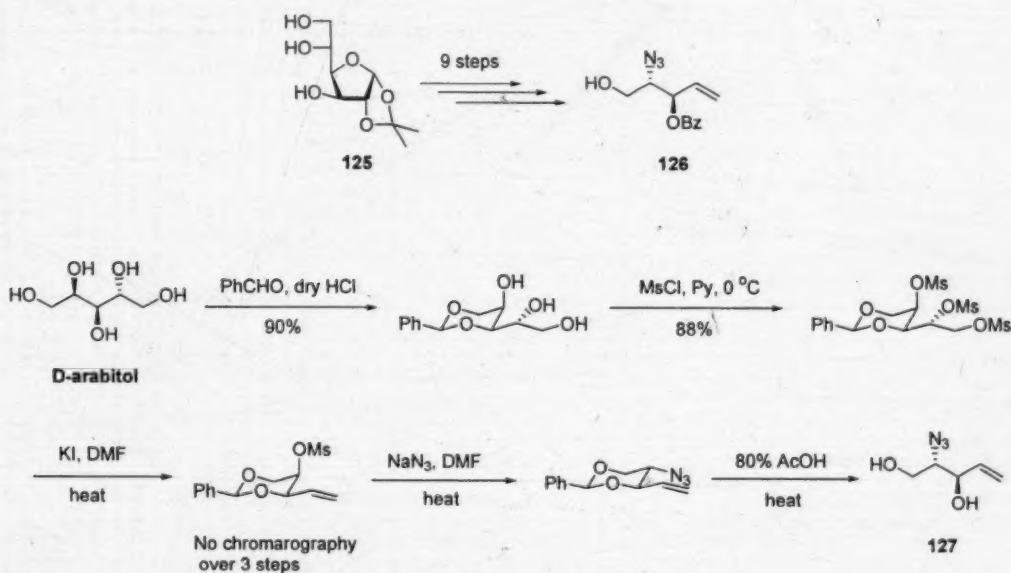


Figure 5.4. Synthesis of truncated sphingosines from **125** and D-arabitol, respectively

* The preparation of this material by Jonathan Cartmell is gratefully acknowledged.

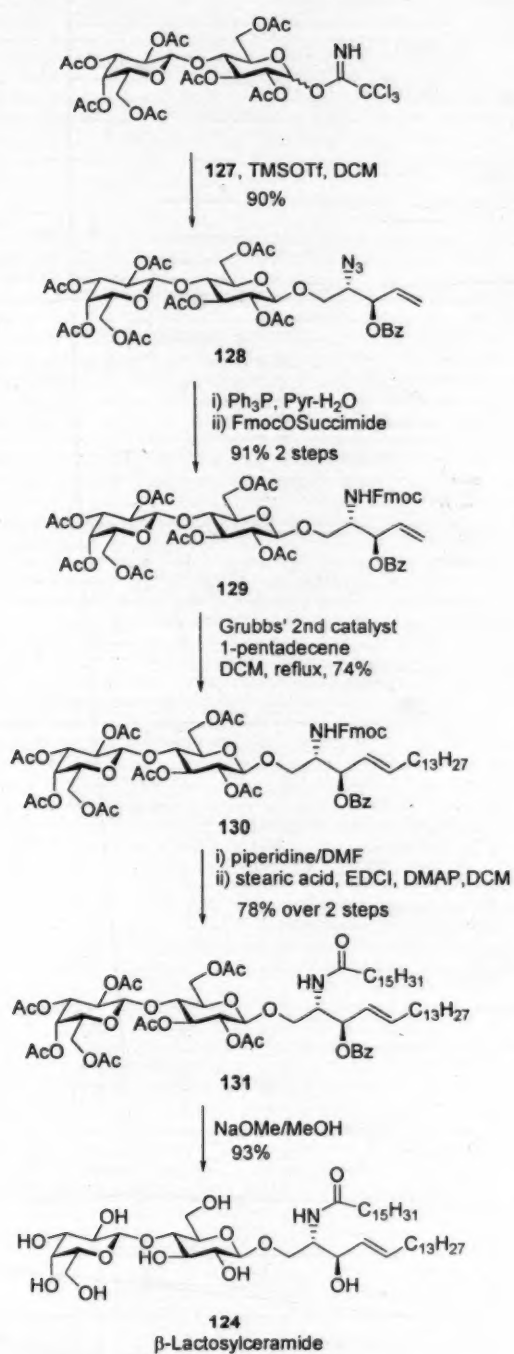
The elaboration of β -lactosylceramide from **126** via olefin cross metathesis is illustrated in Scheme 5.10. First, the glycosylation of **126** with a lactosyl trichloroacetimidate donor in the presence of TMSOTf as the promoter in dichloromethane provided a β -lactosylsphingosine analogue **128** bearing a truncated sphingosine in excellent yield (90%). The β -configuration of **128** was determined by a large $J_{1,2}$ coupling constant (7.8 Hz) between H-1 and H-2 of the glucose moiety. Although the chain elongation of a similar truncated azido-sphingosine via OCM reactions has been accomplished by several groups,^[140c] it did not work well for substrate **128** and the resulting yield was very low, even if a large amount of catalyst and prolonged reaction time were employed. Similar results were also observed by several groups from OCM reactions on some azido-containing substrates.^[140b]

To avoid any complications caused by the presence of azido group, we decided to substitute the azido group with other protected amino functionalities. Therefore, the azido group of **128** was first reduced by triphenylphosphine in a mixture of pyridine and water to give the corresponding amine, which was then reacted directly with FmocOSuccinimide in one-pot manner to provide a Fmoc-protected compound **129** in excellent yield (91%) over two steps. Next, using protocol B in Table 4.1, the chain elongation of **129** was smoothly realized by the reaction with 1-pentadecene, affording the desired β -lactosylsphingosine **130** in 74% yield with excellent *E*-stereoselectivity. The trans double bond of sphingosine moiety in **130** was confirmed by the large trans coupling constant ($J_{4,5} = 15.4$ Hz) between H-4 and H-5 of the sphingosine. After the removal of the Fmoc group by 20% piperidine in DMF, subsequent *N*-acylation with stearic acid in the presence of EDCI and HOBt in dichloromethane was immediately

carried out to give a fully protected β -lactosylceramide **131** in 78% yield over two steps, which was finally deprotected by transesterification condition to furnish the target compound **124** in 93% yield.

5.9 Conclusions

A convergent synthesis of α -galactosylceramide (KRN7000) was successfully developed by an olefin cross metathesis strategy (OCM) through two synthetic routes, A and B (Figure 5.1). The α -galactosylation with an improved yield and excellent anomeric selectivity could be achieved by using tetrabenzyl galactosyl iodide donor and a truncated C-6-phytosphingosine acceptor. Furthermore, this OCM strategy was also applied to the synthesis of β -lactosylceramide, a common core structure in many important GSLs. The successful introduction of OCM in these glycolipids will provide a powerful tool to prepare a series of their analogues and derivatives.



Scheme 5.10. Synthesis of β -lactosylceramide **124** via OCM reaction

Chapter Six

Summary and Future Directions

The research in this thesis describes a new synthetic approach to 2-acetamido-2-deoxy- β -D-glucopyranosides from D-GlcNAc and its extensions to the synthesis of other naturally occurring 2-acetamido-2-deoxy- β -D-hexopyranosides. Additional applications were also explored for the flexible synthesis of phytosphingosines, α -galactosylceramide (KRN7000) and β -lactosylceramide via olefin cross metathesis (OCM) strategy. In addition, an efficient and general synthetic route to several important sphingolipids by using an asymmetric aldol condensation was also developed.

In this research, a novel two-step approach to 2-acetamido-2-deoxy- β -D-glucopyranosides was first developed via a reactive furanose oxazoline donor **5**, which was readily prepared from abundant and inexpensive D-GlcNAc on large scale without the need of chromatography. By using this approach, a series of unprotected 2-acetamido-2-deoxy- β -D-glucopyranosides have been synthesized and can be directly used in the subsequent steps. This mild and stereoselective glycosidation proved to be compatible with a range of functional groups, thereby providing expedient access to the construction of D-GlcNAc-containing complex neoglycoconjugates. The glycosidation mechanism of oxazoline **5** was preliminarily studied and neighboring 2-acetamido group participation is presumably involved in the rearrangement of the furanoside to pyranoside to afford the desirable β -linkage.

The above approach was then applied to the synthesis of other naturally occurring, but expensive, 2-acetamido-2-deoxy- β -D-hexopyranosides and derivatives. Starting from

the versatile building block oxazoline **5** and through suitable manipulation at the C-3 position, three methyl 2-acetamido-2-deoxy- β -D-hexopyranosides (*galacto*-, *allo*- and *gulo*-configurations) and two methyl 2-acetamido-2,4-diamino-2,4,6-trideoxy- β -D-hexopyranosides (*galacto*- and *gulo*-configurations) were prepared in short sequences and high yield. Meanwhile, the biologically important muramic acid and a repeating β -D-GlcNAc-(1 $\rightarrow$ 4)- β -D-MurNAc (NAG-NAM) disaccharide unit of bacterial cell wall peptidoglycan were also efficiently synthesized from oxazoline **5** by the introduction of lactic acid at 3-position. This work further demonstrated the utility of our glycosylation approach and allowed us to prepare expensive 2-amino-2-deoxyhexose derivatives in a concise and practical manner.

With methyl 2-acetamido-2-deoxy- β -D-hexopyranosides available in large quantities, a novel and flexible synthesis of C-18-phytosphingosines was then proposed. In this approach, methyl 2-acetamido-2-deoxy- β -D-hexopyranosides were first converted to the corresponding truncated C-6-phytosphingosine structures via a key reaction of zinc-mediated reductive fragmentation. Then, these truncated structures were rapidly elongated to C-18-phytosphingosines by olefin cross metathesis (OCM). Using this approach, two kinds of unsaturated D-*xylo* and natural D-*ribo*-phytosphingosine (C-18) as well as their saturated counterparts were successfully synthesized. The optimization of olefin cross metathesis (OCM) reaction was also investigated. Fortunately, we first discovered the fact that dropwise addition of Grubbs' second generation catalyst could effectively drive the reaction to completion and good to high yields were usually obtained. This new approach provided us with a general and flexible access to not only

natural C-18-phytosphingosine itself, but also many of its stereoisomers, analogues and derivatives.

Besides the above synthesis of C-18-phytosphingosine by OCM strategy, another efficient approach to (2*S*, 3*R*)-D-*erythro*-sphingolipids by asymmetric aldol condensation was also explored in this research. Several important sphingolipids such as truncated C-5-sphingosine, three sphinganines and two phytosphingosine have been synthesized in three steps from a chiral auxiliary and the appropriate aldehydes. The chain elongation of a truncated sphingosine to C-18-(*E*)-sphingosine by OCM reaction was also demonstrated in this synthesis. This method gave us an alternative synthetic route to some important sphingolipids and analogues.

Following the synthesis of these sphingolipids, a potent immunostimulant α -galactosylceramide (α -GalCer, KRN7000) was efficiently prepared by an OCM strategy via optimal and convergent routes. The highly challenging α -galactosylation could be effectively circumvented by using tetrabenzyl galactosyl iodide as the donor and truncated C-6-phytosphingosine as the acceptor. Excellent glycosylation yield, together with complete α -selectivity, has been obtained. The chain elongations on truncated aglycon moiety were also smoothly accomplished by OCM reaction in high yields using our catalyst addition protocol. Encouraged by the synthesis of α -galactosylceramide (KRN7000), β -lactosylceramide, a common core structure of many GSLs in nature, was also successfully synthesized via OCM reaction with excellent *E*-selectivity. The successful application of the OCM strategy in GSLs will greatly facilitate analogue and derivative synthesis, which are highly required in further biological and medical investigations of GSLs as useful immunomodulatory drugs.

Besides these above completed work, several logical extensions of this research should include:

- (1) Re-investigation needs to be conducted in the synthesis of muramylpeptides to provide the target products with desirable β -pyranoside configuration, as shown in Figure 6.1. In the solid phase approach, a more acid labile trityl chloride resin should be employed rather than Wang resin we used. The resulting muramyloligopeptides could be used as immunoadjuvants and bacterial cell wall biosynthetic precursors.

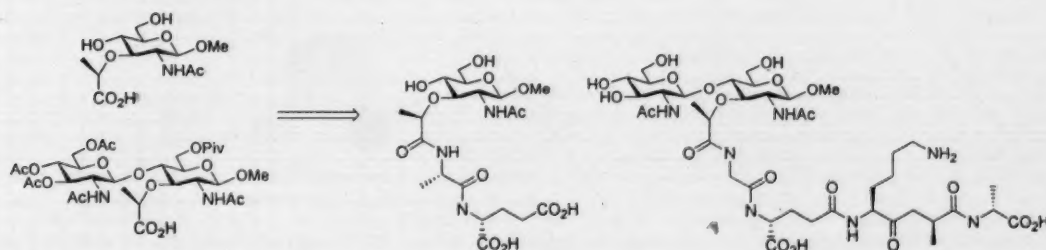


Figure 6.1. Extended synthesis of muramylpeptides

- (2) Since **16** and **19** were readily accessible from D-GlcNAc as described in Chapter three, *L*-lyxo and *L*-arabino-phytosphingosines could also be conveniently synthesized using the approaches we developed in Chapter four (Figure 6.2).

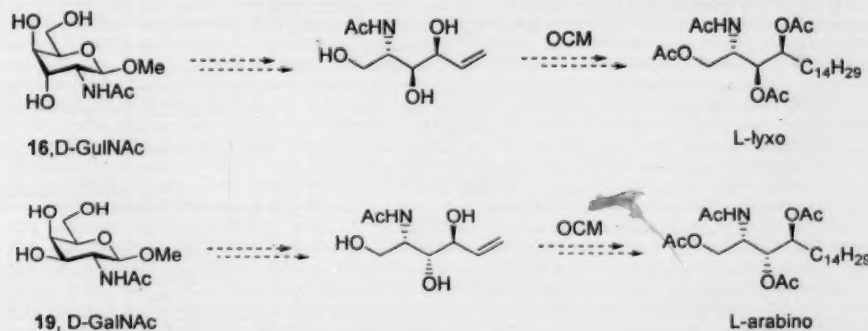


Figure 6.2. Extended synthesis of *L*-arabino and *L*-lyxo phytosphingosines

- (3) The successful synthesis of β -lactosylceramide by OCM strategy will logically result in the efficient and flexible synthesis of complex GM_3 and GM_2 gangliosides when combining with enzymatic methods,^[180-182] as shown in Figure 6.3. More importantly, the sphingosine moiety of gangliosides can be easily modified by OCM and a series of analogues and derivatives will be rapidly synthesized with the objective of developing novel ganglioside-based conjugated cancer vaccines in our group.

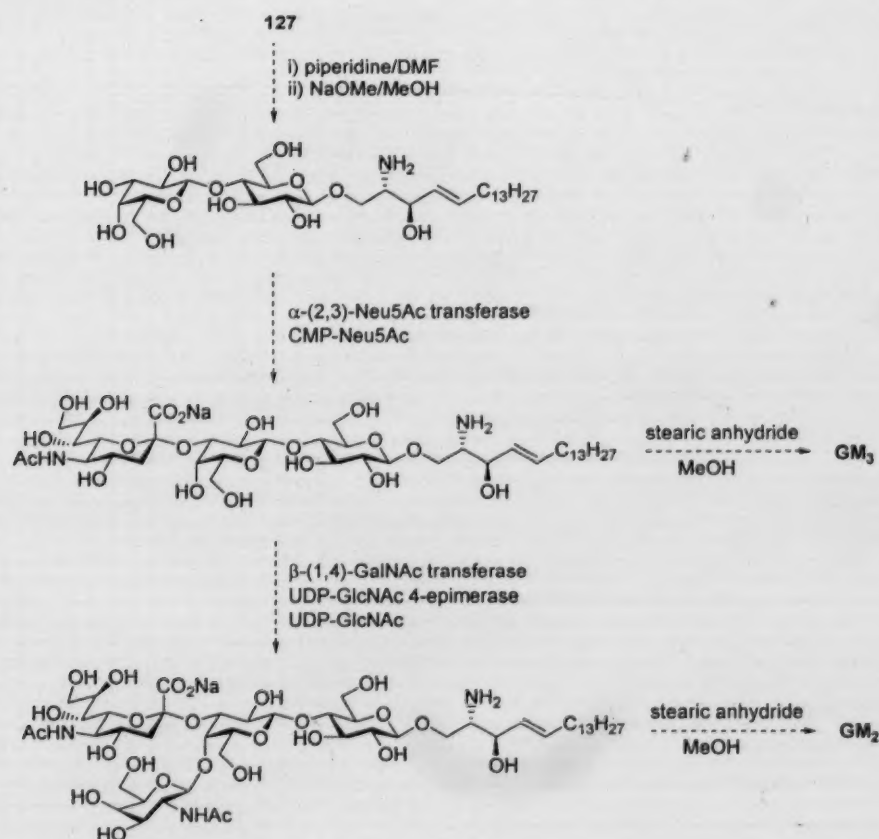


Figure 6.3. Extended chemoenzymatic synthesis of GM_3 and GM_2 gangliosides

In addition to the extensions mentioned above, this research also creates several opportunities for future work.

- (4) The applications of oxazoline **5** as a versatile building block and effective donor need to be further exploited. For example, oxazoline **5** could be employed in the efficient synthesis of some D-GlcNAc containing disaccharides.

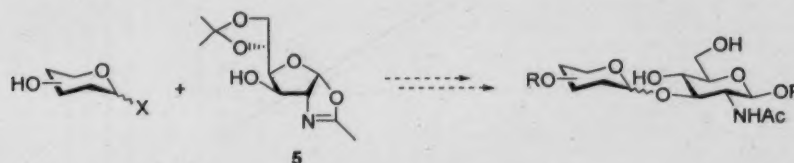


Figure 6.4. Proposed disaccharide synthesis involving oxazoline **5**

- (5) The rapid conversion of acetamido group to tetrazole by treatment with $\text{Trf}_2\text{O}/\text{NaN}_3$ would lead to a new strategy for quick modification of carbohydrates aiming to develop novel inhibitors, probes and other carbohydrate based drugs, as illustrated in Figure 6.5.

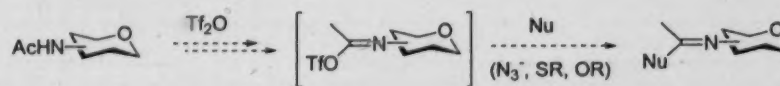


Figure 6.5. Proposed strategy for rapid modification of acetamido-containing carbohydrates

- (6) Truncated C-6-aldehydes and phytosphingosines, which were readily prepared from the corresponding 2-amino-2-deoxy-hexopyranosides via a zinc-mediated reductive fragmentation, are powerful and versatile building blocks and will find broad application in the synthesis of many other interesting bioactive molecules such as azasugars, polyhydroxylated alkaloids and glycosidase inhibitors (Figure 6.6).

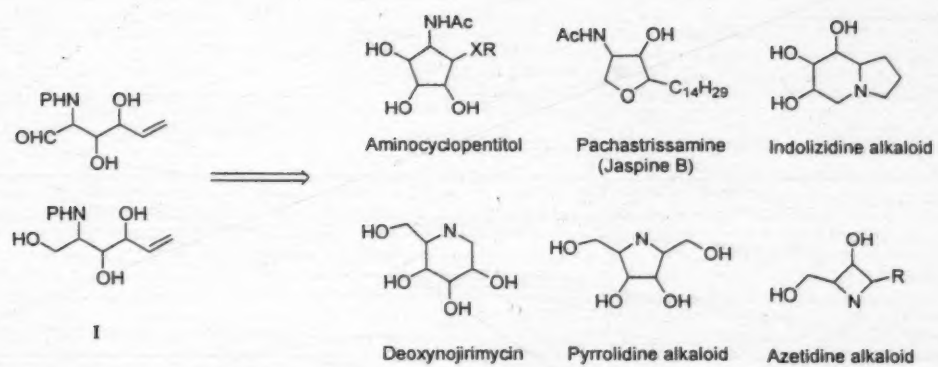


Figure 6.6. Proposed target bioactive molecules synthesized from truncated structure **I**.

Chapter Seven

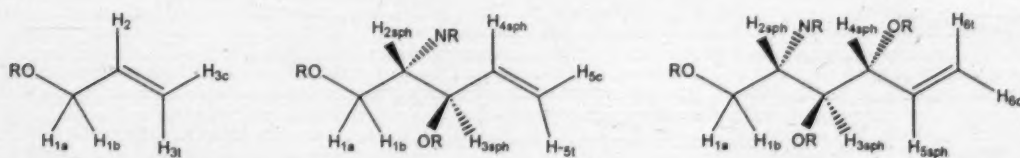
Experimental Section

General Methods:

Chemical synthesis: All chemical reagents were of analytical grade, used as supplied from Sigma-Aldrich unless indicated. Solvents used in organic reactions were distilled under an inert atmosphere, or purified by a solvent purification system made by Innovative Technology Inc., except *N, N*-dimethylformamide, which was stored over 3 Å molecular sieves and subjected to reduced pressure for several minutes prior to use. Unless otherwise noted, all reactions were carried out at room temperature and non-hydrolytic reactions were performed under a positive pressure of argon. Solvents were removed at between 20 and 40 °C (bath temperature) unless indicated. Molecular sieves were stored in an oven (> 170 °C) and cooled *in vacuo* prior to use. Amberlite IR-120 (H⁺ form) was used where acidic ion-exchange resin is indicated. Amberlite IR-400 (Cl form, BDH) was treated with 1M NaOH (3x) and followed by washing with methanol (three times) affording basic ion-exchange resin. All sonication were performed in a Branson B-32H (50/60 Hz) sonic bath. Zinc dust (Aldrich 20.988-8) was activated immediately before use: zinc (5 g) in 1 M aqueous HCl (50 mL) was stirred at RT for 10-15 min, then filtered, and washed with water (3 x 30 mL) and ethyl ether (2 x 50 mL). Analytical thin layer chromatography (TLC) was conducted on silica gel 60-F₂₅₄ (Merck). Plates were visualized under UV light, and/or by treatment with either acidic cerium ammonium molybdate or 5% ethanolic sulfuric acid, followed by heating.

The solid phase synthesis of muramylpeptides was carried out by either manually or by machine (Applied systems 433A peptide synthesizer). Fmoc-D-Ala was purchased from NOVAbiochem and Fmoc-D-Glu-OtBu was from AnaSpec Inc. Wang resin and coupling reagents were also supplied from NOVAbiochem. HPLC purification was conducted using a Waters Delta 600 with a UV absorbance detector (Waters 2996 photodiode array). Separations were performed on Phenomenex Luna C₁₈-silica reverse-phase preparative columns with combinations of water and acetonitrile containing 0.1% trifluoroacetic acid as eluent.

Characterization: ¹H NMR spectra were recorded on Varian INOVA 400, 500 or 600 MHz spectrometers and ¹³C NMR spectra were recorded at 125 MHz. ¹H NMR chemical shifts are reported in δ (ppm) units using signals from residual solvents as reference. ¹³C NMR chemical shifts are referenced to internal solvent or to external acetone at δ 2.25 ppm in the case of D₂O. ¹H and ¹³C NMR spectra were tentatively assigned with the assistance of COSY, HMQC, HMBC, TOCSY and TROESY spectra as necessary. NMR data for compounds containing allyl group, sphingosine, phytosphingosine or ceramide and derivatives as the aglycons were labeled as indicated below.

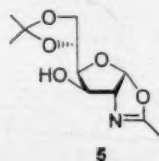


Electrospray ionization (ESI) mass spectra were recorded on a Micromass Zabspec TOF mass spectrometer. For high resolution mass determination, spectra were obtained by voltage scan over a narrow range at a resolution of approximately 10⁴. MALDI TOF mass spectra were recorded on a Voyager Elite spectrometer from Applied

Biosystems. All spectra were acquired at the mass spectrometry laboratory of this department. Optical rotations were determined with a Perkin-Elmer model 241 polarimeter for samples in a 10 cm cell at 22 ± 2 °C, and are reported in units of $\text{degr. mL g}^{-1} \text{ dm}^{-1}$. Elemental analysis and optical rotations were performed by the analytical services facility of this department.

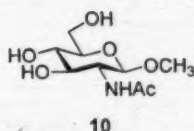
Chapter Two

Synthesis of 2-Acetamido-2-deoxy- β -D-glucopyranosides via a Furanose Oxazoline Derivative



2-Methyl-(1,2-dideoxy-5,6-*O*-isopropylidene- α -D-glucofurano)-[2,1-d]-2-oxazoline (**5**)

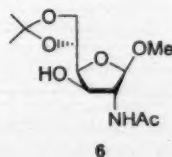
Oxazoline **5** was prepared according to a literature procedure ^[74] on a large scale (30 g). The ^1H and ^{13}C NMR spectra are identical to those reported. ^1H NMR (600 MHz, CDCl_3) δ 6.14 (d, 1H, $J_{1,2} = 5.1$ Hz, H-1), 4.42 (dd, 1H, $J_{2,3} = 5.1$ Hz, H-2), 4.37 (d, 1H, $J_{3,4} = 2.9$ Hz, H-3), 4.31 (ddd, 1H, $J_{5,6a} = 6.1$ Hz, $J_{5,6b} = 4.8$ Hz, H-5), 4.12 (dd, 1H, $J_{6a,6b} = 8.7$ Hz, H-6a), 4.00 (dd, 1H, H-6b), 3.73 (dd, 1H, $J_{4,5} = 7.9$ Hz, H-4), 2.01 (s, 3H, NHCOCH_3), 1.40 (s, 3H, $\text{C}(\text{CH}_3)_2$), 1.33 (s, 3H, $\text{C}(\text{CH}_3)_2$); ^{13}C NMR (125 MHz, CDCl_3) δ 167.1, 109.4, 107.1, 81.8, 78.2, 74.8, 72.9, 67.4, 26.8, 25.2, 14.1.



Methyl 2-acetamido-2-deoxy- β -D-glucopyranoside (10)

Oxazoline **5** (20.0 g) was dissolved in anhydrous methanol (500 mL) and *p*-TsOH (0.2 equiv) was added. The mixture was stirred at RT for 5 h. The reaction was monitored by TLC and quenched with Et₃N to adjust the pH to 7.5. The solvent was removed under reduced pressure and the residue was washed with CH₂Cl₂ for several times to afford a white and pure solid (~19.0 g, yield 98%). [α]_D -39 (*c* 1.1, CH₃OH); ¹H NMR (500 MHz, D₂O): δ 4.44 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1), 3.93 (dd, 1H, $J_{6a,6b}$ = 12.2 Hz, H-6a), 3.76 (dd, 1H, 5.6 Hz, H-6b), 3.69 (dd, 1H, $J_{2,3}$ = 10.2 Hz, H-2), 3.54 (dd, 1H, $J_{3,4}$ = 8.5 Hz, H-3), 3.50 (s, 3H, OCH₃), 3.46 (ddd, 1H, $J_{5,6a}$ = 5.6 Hz, $J_{5,6b}$ = 2.2 Hz, H-5), 3.43 (dd, 1H, $J_{4,5}$ = 10.0 Hz, H-4), 2.03 (s, 3H, NHCOCH₃); ¹³C NMR (125 MHz, D₂O): δ 175.56, 102.81, 76.77, 74.84, 70.83, 61.63, 57.93, 56.33, 23.03; ESI HRMS *m/z* calcd. for C₉H₁₇NO₆Na (M+Na⁺): 258.0948. Found 258.0948.

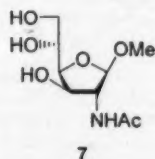
If the reaction was quenched with Dowex-2 (OH⁻) before it went to completion, compounds **6** and **7** could be isolated.



Methyl 2-acetamido-2-deoxy-5,6-*O*-isopropylidene- β -D-glucofuranoside (6)

[α]_D -36 (*c* 0.9, CH₃OH); {lit.^[74] [α]_D -37 (*c* 1.2, CH₃OH)}; ¹H NMR (500 MHz, CDCl₃): δ 5.51 (d, 1H, $J_{NH,2}$ = 6.1 Hz, NH), 4.84 (s, 1H, H-1), 4.39 (ddd, 1H, $J_{5,6a}$ = 5.7

Hz, $J_{5,6b} = 5.7$ Hz, H-5), 4.32 (d, 1H, $J = 7.0$ Hz, H-2), 4.21 (d, 1H, $J_{3,4} = 4.9$ Hz, H-3), 4.16 (dd, 1H, $J_{6a,6b} = 8.5$ Hz, H-6a), 4.14 (dd, 1H, $J_{4,5} = 7.6$ Hz, H-4), 4.02 (dd, 1H, $J = 8.6$ Hz, H-6b), 3.39 (s, 3H, OCH₃), 1.98 (s, 3H, NHCOCH₃), 1.44 (s, 3H, C(CH₃)₂), 1.37 (s, 3H, C(CH₃)₂); ¹³C NMR (125M Hz, CDCl₃): δ 169.9, 109.3, 107.7, 83.3, 77.3, 77.0, 76.7, 75.2, 74.0, 67.0, 61.9, 55.6, 26.8, 25.2, 23.2; ESI HRMS m/z calcd. for C₁₂H₂₁NO₆Na (M+Na⁺): 298.1261. Found 298.1258.



Methyl 2-acetamido-2-deoxy- β -D-glucopyranoside (7)

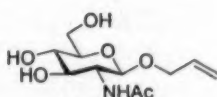
$[\alpha]_D -54$ (c 1, CH₃OH); {lit.^[74] $[\alpha]_D -56$ (c 1, CH₃OH)}; ¹H NMR (500 MHz, CD₃OD): δ 4.77 (d, 1H, $J_{1,2} = 1.0$ Hz, H-1), 4.17 (dd, 1H, $J_{3,4} = 4.8$ Hz, H-3), 4.13 (d, 1H, $J_{2,3} = 1.7$ Hz, H-2), 4.05 (dd, 1H, $J_{4,5} = 8.8$ Hz, H-4), 3.94 (ddd, 1H, $J_{5,6a} = 3.0$ Hz, $J_{5,6b} = 5.9$ Hz, H-5), 3.82 (dd, 1H, $J_{6a,6b} = 11.5$ Hz, H-6a), 3.64 (dd, 1H, H-6b), 3.34 (s, 3H, OCH₃), 1.96 (s, 3H, NHCOCH₃); ¹³C NMR (125 MHz, CD₃OD): δ 173.2, 109.3, 82.5, 76.1, 71.8, 65.2, 64.1, 55.6, 22.4; ESI HRMS m/z calcd. for C₉H₁₇NO₆Na (M+Na⁺): 258.0948. Found 258.0947.

General procedure A for β -glycosidation of oxazoline 5 (Table 2.1 and 2.2)

To a mixture of oxazoline 5 (1.0 mmol) in anhydrous alcohol (2.0 mL) or in a mixture of anhydrous alcohol (1 mL) and CH₂Cl₂ (1 mL) containing molecular sieves (4 Å, 300 mg), anhydrous *p*-TsOH or CSA (0.2-1 equiv) was added. The mixture was stirred at RT for 18

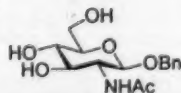
h. When the TLC indicated that reaction was finished, Dowex 2 (OH⁻) resin was added to quench the reaction until the pH reach 7.0. After filtration and solvent removal, the residue was either washed with CH₂Cl₂ several times or purified by flash chromatography (CH₂Cl₂: CH₃OH 10:1) to afford the desired product as a white solid.

(* All compounds from Table 2.1 and 2.2 are listed in below without numbers)



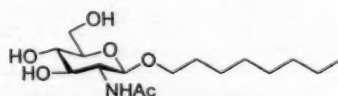
Allyl 2-acetamido-2-deoxy- β -D-glucopyranoside

The title compound (931 mg) was prepared from oxazoline **5** and allyl alcohol according to the general procedure A and purified by washing with dichloromethane. Yield 71%; if purified by chromatography, the yield was 82%. $[\alpha]_D -25$ (*c* 1.0, CH₃OH); ¹H NMR (500 MHz, D₂O) δ 5.90 (dddd, 1H, $J_{2,3t} = 17.3$ Hz, $J_{2,3c} = 10.5$ Hz, H-2_{allyl}), 5.30 (ddd, 1H, $J_{3t,3c} = 1.6$ Hz, H-3_{tallyl}), 5.26 (dd, 1H, H-3_{callyl}), 4.56 (d, 1H, $J_{1,2} = 8.5$ Hz, H-1), 4.33 (dd, 1H, $J_{1a,1b} = 13.2$ Hz, $J_{1a,2} = 5.2$ Hz, H-1_{aallyl}), 4.16 (dd, 1H, $J_{1b,2} = 6.3$ Hz, H-1_{ballyl}), 3.95 (dd, 1H, $J_{6a,6b} = 12.4$ Hz, H-6_a), 3.74 (m, 1H, H-6_b), 3.70 (dd, 1H, $J_{2,3} = 10.3$ Hz, H-2), 3.53 (m, 1H, H-3), 3.41-3.47 (m, 2H, H-4 and H-5), 2.02 (s, 3H, NHCOCH₃); ¹³C NMR (125 MHz, D₂O): δ 175.5, 134.2, 119.0, 100.9, 76.8, 74.8, 71.3, 70.8, 61.6, 56.4, 32.0; ESI HRMS *m/z* calcd. for C₁₁H₁₉NO₆Na (M+Na⁺): 284.1105. Found 284.1105.



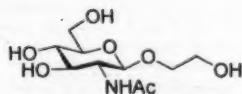
Benzyl 2-acetamido-2-deoxy- β -D-glucopyranoside

The title compound (955 mg) was prepared from oxazoline **5** and benzyl alcohol according to the general procedure A, and purified by washing with dichloromethane. Yield 60%; if purified by chromatography, the yield was 73%. $[\alpha]_D -29$ (c 0.9, CH₃OH); ¹H NMR (600 MHz, D₂O) δ 7.43-7.50 (m, 3H, ArH), 7.40 (m, 2H, ArH), 4.90 (d, 1H, J = 12.2 Hz, PhCH₂), 4.69 (d, 1H, J = 12.2 Hz, PhCH₂), 4.55 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1), 3.97 (dd, 1H, J = 12.4 Hz, 0.9 Hz, H-6a), 3.79 (m, 1H, H-6b), 3.72 (dd, 1H, $J_{2,3}$ = 9.0 Hz, H-2), 3.45-3.52 (m, 3H, H-3, H-4 and H-5), 1.95 (s, 3H, NHCOCH₃); ¹³C NMR (125 MHz, D₂O): δ 175.4, 137.7, 129.6, 129.5, 129.3, 100.8, 76.8, 74.6, 72.3, 70.8, 61.7, 56.4, 23.0; ESI HRMS m/z calcd. for C₁₅H₂₁NO₆Na (M+Na⁺): 334.1261. Found 334.1257.



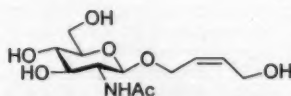
Octyl 2-acetamido-2-deoxy- β -D-glucopyranoside

The title compound (206 mg) was prepared from oxazoline **5** and octyl alcohol according to the general procedure A and purified by chromatography. Yield 69%. $[\alpha]_D -22.8$ (c 1.0, CH₃OH); ¹H NMR (500 MHz, CD₃OD): δ 4.38 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1), 3.84-3.90 (m, 2H, H-6a and OCH_aCH_b), 3.67 (dd, 1H, $J_{6b,6a}$ = 11.9 Hz, H-6b), 3.62 (dd, 1H, $J_{2,3}$ = 10.3 Hz, H-2), 3.42-3.47 (m, 2H, H-3 and OCH_aCH_b), 3.30 (d, 1H, $J_{4,5}$ = 9.1 Hz, H-4), 3.24 (ddd, 1H, $J_{5,6b}$ = 5.7 Hz, $J_{5,6a}$ = 2.3 Hz, H-5), 1.96 (s, 3H, NHCOCH₃), 1.50-1.56 (m, 2H, CH₂), 1.25-1.36 (m, 10H, octyl CH₂), 0.89 (t, 3H, J = 6.8 Hz, octyl CH₃); ¹³C NMR (125 MHz, CD₃OD): δ 173.6, 102.7, 77.7, 76.1, 70.6, 62.8, 57.5, 49.5, 33.0, 30.7, 30.5, 30.5, 27.2, 23.7, 23.0, 14.4; ESI HRMS m/z calcd. for C₁₆H₃₁NO₆Na (M+Na⁺): 356.2044. Found 356.2045.



2-Hydroxyethyl 2-acetamido-2-deoxy- β -D-glucopyranoside

The title compound (216 mg) was prepared from oxazoline **5** and 2-hydroxyethanol according to the general procedure A and purified by chromatography. Yield 78%. $[\alpha]_D - 15.6$ (*c* 1.0, CH₃OH); ¹H NMR (600 MHz, D₂O): δ 4.55 (d, 1H, $J_{1,2} = 8.5$ Hz, H-1), 3.90-3.94 (m, 2H, H-6a and OCH_aCH_b), 3.69-3.76 (m, 5H, H-2, H-6b, OCH_aCH_b, OCH_aCH_bCH_cH_dOH and OCH_aCH_bCH_cH_dOH), 3.54 (m, 1H, H-3), 3.42-4.38 (m, 2H, H-4 and H-5), 2.12 (s, 3H, NHCOCH₃); ¹³C NMR (125 MHz, D₂O): δ 175.7, 102.1, 76.7, 74.7, 72.0, 70.8, 61.6, 61.5, 56.4, 23.0; ESI HRMS *m/z* calcd. for C₁₀H₁₉NO₇Na (M+Na⁺): 288.1054. Found 288.1053.

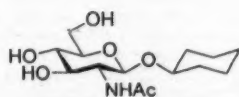


4-Hydroxy-(*Z*)-but-2-enyl 2-acetamido-2-deoxy- β -D-glucopyranoside

The title compound (214 mg) was prepared from oxazoline **5** and *cis*-but-2-en-1,4-diol according to the general procedure A and purified by chromatography. Yield 77%. $[\alpha]_D - 19.5$ (*c* 1.0, CH₃OH); ¹H NMR (500 MHz, D₂O): δ 5.84 (m, 1H, alkene), 5.67 (m, 1H, alkene), 4.54 (d, 1H, $J_{1,2} = 8.5$ Hz, H-1), 4.35 (m, 1H, OCH_aH_bCH=CH), 4.30 (m, 1H, OCH_aH_bCH=CH), 4.15-4.20 (m, 2H, CH=CHCH₂OH), 3.92 (dd, 1H, $J_{6a,6b} = 12.4$ Hz, $J_{6a,5} = 1.3$ Hz, H-6a), 3.74 (m, 1H, H-6b), 3.68 (dd, 1H, $J_{2,3} = 10.3$ Hz, H-2), 3.53 (m, 1H, H-3), 3.42-3.47 (m, 2H, H-4 and H-5), 2.03 (s, 3H, NHCOCH₃); ¹³C NMR (125

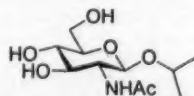
MHz, D₂O): δ 175.5, 133.7, 127.5, 100.7, 76.8, 74.7, 70.8, 65.3, 61.6, 58.0, 56.4, 23.0;

ESI HRMS m/z calcd. for C₁₂H₂₁NO₇Na (M+Na⁺): 314.1210. Found 314.1209.



Cyclohexyl 2-acetamido-2-deoxy- β -D-glucopyranoside

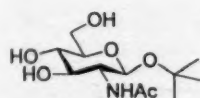
The title compound (299 mg) was prepared from oxazoline **5** and cyclohexanol according to the general procedure A and purified by chromatography. Yield 73%. $[\alpha]_D -8$ (c 0.9, CH₃OH); ¹H NMR (500 MHz, D₂O): δ 4.62 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1), 3.91 (dd, 1H, $J_{6a,6b}$ = 12.3 Hz, $J_{6a,5}$ = 1.6 Hz, H-6a), 3.72-3.80 (m, 2H, H-6b and OCH), 3.65 (dd, 1H, $J_{2,3}$ = 8.4 Hz, H-2), 3.55 (m, 1H, H-3), 3.41-3.47 (m, 2H, H-4 and H-5), 2.02 (s, 3H, NHCOCH₃), 1.20-1.90 (m, 10H, cyclohexyl-H); ¹³C NMR (125 MHz, D₂O): δ 175.7, 102.2, 76.8, 74.5, 71.1, 70.7, 61.6, 56.4, 44.3, 23.1; ESI HRMS m/z calcd. for C₁₄H₂₅NO₆Na (M+Na⁺): 326.1574. Found 326.1573.



Isopropyl 2-acetamido-2-deoxy- β -D-glucopyranoside

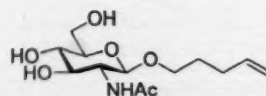
The title compound (908 mg) was obtained as a mixture contaminated with α -isomer (α/β : 1/6) from oxazoline **5** and isopropyl alcohol according to the general procedure A, and purified by washing with dichloromethane. Yield 77%; if purified by chromatography, the yield was 66%. ¹H NMR (500 MHz, D₂O): δ 4.59 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1), 4.01 (sept, 1H, J = 6.2 Hz, OCHMe₂), 3.91 (dd, 1H, $J_{6a,6b}$ = 12.2 Hz, $J_{6a,5}$ = 2.2

Hz, H-6a), 3.73 (dd, 1H, $J_{6b,5} = 5.6$ Hz, H-6b), 3.62 (dd, 1H, $J_{2,3} = 10.4$ Hz, H-2), 3.53 (m, 1H, H-3), 3.39-3.46 (m, 2H, H-4 and H-5), 2.03 (s, 3H, NHCOCH_3), 1.19 (d, 3H, $J = 6.2$ Hz, $\text{C}(\text{CH}_3)_2$), 1.12 (d, 3H, $J = 6.2$ Hz, $\text{C}(\text{CH}_3)_2$); ^{13}C NMR (125 MHz, D_2O): δ 175.4, 100.5, 76.7, 74.8, 74.5, 70.8, 61.8, 56.7, 23.2, 23.0, 22.0; ESI HRMS m/z Calcd. for $\text{C}_{11}\text{H}_{21}\text{NO}_6\text{Na}$ ($\text{M}+\text{Na}^+$): 286.1261. Found, 286.1264.



***t*-Butyl 2-acetamido-2-deoxy- β -D-glucopyranoside**

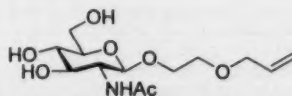
The title compound (134 mg) was obtained from oxazoline **5** and *t*-butyl alcohol by chromatography as a mixture contaminated with α -isomer (α/β : 1/4) according to the general procedure A. Yield 51%. ^1H NMR (500 MHz, D_2O): δ 4.66 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1), 3.87 (dd, 1H, $J_{6a,6b} = 12.3$ Hz, H-6a), 3.69 (dd, 1H, H-6b), 3.60 (dd, 1H, $J_{2,3} = 10.4$ Hz, H-2), 3.55 (dd, 1H, $J_{3,4} = 8.1$ Hz, H-3), 3.43 (ddd, 1H, $J_{5,6a} = 5.9$ Hz, $J_{5,6b} = 2.3$ Hz, H-5), 3.39 (dd, 1H, $J_{4,5} = 9.9$ Hz, H-4), 2.03 (s, 3H, NHCOCH_3), 1.22 (s, 9H, $\text{C}(\text{CH}_3)_3$); ^{13}C NMR (125 MHz, D_2O): δ 175.3, 96.4, 78.2, 76.4, 74.7, 71.0, 61.8, 56.9, 28.4, 23.1; ESI HRMS m/z calcd. for $\text{C}_{12}\text{H}_{23}\text{NO}_6\text{Na}$ ($\text{M}+\text{Na}^+$): 300.1418. Found 300.1419.



***n*-Pentenyl 2-acetamido-2-deoxy- β -D-glucopyranoside**

The title compound (846 mg) was prepared from oxazoline **5** and *n*-pentenyl alcohol according to the general procedure A, and purified by washing with dichloromethane.

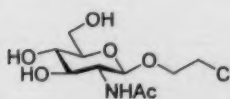
Yield 64%; if purified by chromatography, the yield was 76%. $[\alpha]_D -27$ (c 0.9, CH_3OH); ^1H NMR (600 MHz, D_2O): δ 5.90 (dddd, 1H, $J_{4,5t} = 17.3$ Hz, $J_{4,5c} = 10.3$ Hz, $J_{4,3a} = J_{4,3b} = 6.7$ Hz, H-4_{pent}), 5.08 (dd, 1H, $J_{5t,5c} = 1.6$ Hz, H-5t_{pent}), 5.02 (dd, 1H, H-5c_{pent}), 4.51 (d, 1H, $J_{1,2} = 8.5$ Hz, H-1), 3.88-3.94 (m, 2H, H-6a and H-1a_{pent}), 3.74 (m, 1H, H-6b), 3.68 (dd, 1H, $J_{2,3} = 10.4$ Hz, H-2), 3.61 (dt, 1H, $J_{1a,1b} = 10.3$ Hz, $J_{1b,2} = 6.4$ Hz, H-1b_{pent}), 3.54 (m, 1H, H-3), 3.41-3.46 (m, 2H, H-4 and H-5), 2.05-2.12 (m, 2H, H-3_{pent}), 2.04 (s, 3H, NHCOCH_3), 1.65 (m, 2H, H-2_{pent}); ^{13}C NMR (125 MHz, D_2O): δ 175.4, 139.6, 115.7, 102.0, 76.7, 74.7, 70.8, 70.5, 61.6, 56.5, 30.1, 28.7, 23.1; ESI HRMS m/z calcd. for $\text{C}_{13}\text{H}_{23}\text{NO}_6\text{Na}$ ($\text{M}+\text{Na}^+$): 312.1417. Found 312.1416.



2-Allyloxyethyl 2-acetamido-2-deoxy- β -D-glucopyranoside

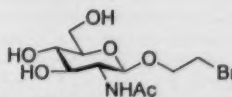
The title compound (226 mg) was prepared from oxazoline **5** and 2-allyloxyethanol according to the general procedure A and purified by chromatography. Yield 74%. $[\alpha]_D -24$ (c 1.0, CH_3OH); ^1H NMR (500 MHz, D_2O): δ 5.93 (dddd, 1H, $J = 17.3$ Hz, 10.5 Hz, 5.9 Hz, 5.9 Hz, allyl-H), 5.32 (dddd, 1H, $J = 17.3$ Hz, 3.2 Hz, 1.6 Hz, 1.6 Hz, allyl-H), 5.26 (dddd, 1H, $J = 10.5$ Hz, 2.5 Hz, 1.5 Hz, 1.5 Hz, allyl-H), 4.55 (d, 1H, $J_{1,2} = 8.5$ Hz, H-1), 4.03-4.06 (ddd, 2H, $J = 5.9$ Hz, 1.3 Hz, 1.3 Hz, allyl-H), 4.00 (ddd, 1H, $J = 11.8$ Hz, 5.7 Hz, 2.9 Hz, $\text{OCH}_a\text{H}_b\text{CH}_c\text{H}_d\text{O}$), 3.92 (dd, 1H, $J_{6a,6b} = 12.2$ Hz, $J = 1.5$ Hz, H-6a), 3.62-3.78 (m, 5H, H-2, H-6b, $\text{OCH}_a\text{H}_b\text{CH}_c\text{H}_d\text{O}$, $\text{OCH}_a\text{H}_b\text{CH}_c\text{H}_d\text{O}$, $\text{OCH}_a\text{H}_b\text{CH}_c\text{H}_d\text{O}$), 3.53 (m, 1H, H-3), 3.41-3.46 (m, 2H, H-4 and H-5), 2.03 (s, 3H, NHCOCH_3); ^{13}C NMR

(125 MHz, D₂O): δ 175.4, 134.6, 119.0, 101.9, 76.7, 74.7, 72.6, 70.8, 69.8, 69.6, 56.4, 23.1; ESI HRMS m/z calcd. for C₁₃H₂₃NO₇Na (M+Na⁺): 328.1367. Found 328.1369.



2-Chloroethyl 2-acetamido-2-deoxy- β -D-glucopyranoside

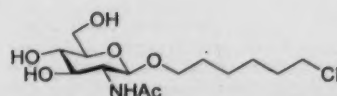
The title compound (168 mg) was prepared from oxazoline **5** and 2-chloroethanol according to the general procedure A and purified by chromatography. Yield 65%. $[\alpha]_D - 22$ (c 0.9, CH₃OH); ¹H NMR (500 MHz, D₂O): δ 4.58 (d, 1H, $J_{1,2} = 8.5$ Hz, H-1), 4.12 (ddd, 1H, $J = 12.0$ Hz, 5.8 Hz, 3.7 Hz, OCH_aH_bCH_cH_dCl), 3.92 (dd, 1H, $J_{6a,6b} = 12.0$ Hz, H-6a), 3.88 (ddd, 1H, $J = 12.0$ Hz, 7.0 Hz, 3.7 Hz, OCH_aH_bCH_cH_dCl), 3.66-3.76 (m, 4H, H-2, H-6b, OCH_aH_bCH_cH_dCl and OCH_aH_bCH_cH_dCl), 3.55 (m, 1H, H-3), 3.42-3.48 (m, 2H, H-4 and H-5), 2.02 (s, 3H, NHCOCH₃); ¹³C NMR (500 MHz, D₂O): δ 175.7, 102.2, 76.8, 71.1, 70.8, 61.6, 56.4, 44.3, 23.1; ESI HRMS m/z calcd. for C₁₀H₁₈NO₆ClNa (M+Na⁺): 306.0715. Found 306.0715.



2-Bromoethyl 2-acetamido-2-deoxy- β -D-glucopyranoside

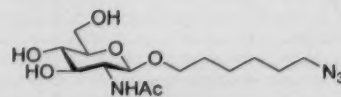
The title compound (181 mg) was prepared from oxazoline **5** and 2-bromoethanol according to the general procedure A and purified by chromatography. Yield 63%. $[\alpha]_D - 9.4$ (c 1.1, CH₃OH); ¹H NMR (500 MHz, D₂O): δ 4.57 (d, 1H, $J_{1,2} = 8.5$ Hz, H-1), 4.18 (ddd, 1H, $J = 12.2$ Hz, 6.0 Hz, 4.0 Hz, OCH_aH_bCH_cH_dBr), 3.88-3.94 (m, 2H,

OCH_aH_bCH_cH_dBr and H-6a), 3.73 (m, 1H, H-6b), 3.70 (dd, 1H, $J_{2,3} = 10.4$ Hz, H-2), 3.49-3.60 (m, 3H, H-3, OCH_aH_bCH_cH_dBr and OCH_aH_bCH_cH_dBr), 3.41-3.47 (m, 2H, H-4 and H-5), 2.04 (s, 3H, NHCOCH₃); ¹³C NMR (125 MHz, D₂O): δ 175.6, 102.1, 76.8, 74.5, 70.8, 70.7, 61.6, 56.3, 32.3, 23.3; ESI HRMS m/z calcd. for C₁₀H₁₈NO₆NaBr (M+Na⁺): 350.0210. Found 350.0206.



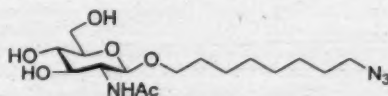
6-Chlorohexyl 2-acetamido-2-deoxy- β -D-glucopyranoside

The title compound (216 mg) was prepared from oxazoline **5** and 6-chlorohexanol according to the general procedure A and purified by chromatography. Yield 72%. $[\alpha]_D - 20$ (c 0.7, CH₃OH:CHCl₃ = 1:1, v/v); ¹H NMR (600 MHz, D₂O): δ 4.50 (d, 1H, $J_{1,2} = 8.5$ Hz, H-1), 3.92 (dd, 1H, $J = <1.0$ Hz, 13.4 Hz, H-6a), 3.90 (ddd, 1H, $J = 10.5$ Hz, 6.0 Hz, 6.0 Hz, OCH_aH_b), 3.74 (m, 1H, H-6b), 3.67 (dd, 1H, $J_{2,3} = 10.4$ Hz, H-2), 3.63 (t, 2H, $J = 6.7$ Hz, CH₂Cl), 3.60 (ddd, 1H, $J = 10.2$ Hz, 6.4 Hz, 6.4 Hz, OCH_aH_b), 3.54 (m, 1H, H-3), 3.41-3.44 (m, 2H, H-4 and H-5), 2.04 (s, 3H, NHCOCH₃), 1.74-1.80 (m, 2H, CH₂), 1.54-1.60 (m, 2H, CH₂), 1.31-1.43 (m, 4H, 2 $\times$ CH₂); ¹³C NMR (125 MHz, D₂O): δ 175.3, 102.0, 76.7, 74.7, 71.2, 70.8, 61.7, 56.5, 46.6, 32.7, 29.3, 26.5, 25.2, 23.1; ESI HRMS m/z calcd. for C₁₄H₂₆NO₆ClNa (M+Na⁺): 362.1341. Found 362.1342.



6-Azidohexyl 2-acetamido-2-deoxy- β -D-glucopyranoside

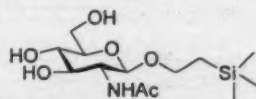
The title compound (204 mg) was prepared from oxazoline **5** and 6-azidoheptanol according to the general procedure A and purified by chromatography. Yield 70%. $[\alpha]_D -15$ (*c* 0.8, CH₃OH); ¹H NMR (600 MHz, D₂O): δ 4.50 (d, 1H, $J_{1,2} = 8.5$ Hz, H-1), 3.92 (dd, 1H, $J_{6a,6b} = 12.1$ Hz, H-6a), 3.90 (ddd, 1H, $J = 12.0$ Hz, 6.0 Hz, 6.0 Hz, OCH₂H_b), 3.74 (m, 1H, H-6b), 3.67 (dd, 1H, $J_{2,3} = 10.3$ Hz, H-2), 3.60 (ddd, 1H, $J = 10.2$ Hz, 6.5 Hz, 6.5 Hz, OCH₂H_b), 3.53 (m, 1H, H-3), 3.41-3.47 (m, 2H, H-4 and H-5), 3.32 (t, 2H, $J = 6.9$ Hz, CH₂N₃), 1.54-1.6m (m, 4H, 2 $\times$ CH₂), 1.31-1.41 (m, 4H, 2 $\times$ CH₂), 2.04 (s, 3H, NHCOCH₃); ¹³C NMR (125 MHz, D₂O): δ 175.3, 102.0, 76.7, 74.7, 71.2, 70.8, 61.7, 56.5, 52.0, 29.3, 28.8, 26.5, 25.5, 23.1; ESI HRMS *m/z* calcd. for C₁₄H₂₆N₄O₆Na (M+Na⁺): 369.1745. Found 369.1747.



8-azido-octyl 2-acetamido-2-deoxy- β -D-glucopyranoside

The title compound (265 mg) was prepared from oxazoline **5** and 8-azidoheptanol according to the general procedure A and purified by chromatography. Yield 67%. $R_f = 0.45$ (5:1 CH₂Cl₂-CH₃OH); $[\alpha]_D -18.5$ (*c* 0.9, CH₃OH); ¹H NMR (600 MHz, D₂O): δ 4.50 (d, 1H, $J_{1,2} = 8.4$ Hz, H-1), 3.91 (d, 1H, $J_{6a,6b} = 12.1$ Hz, $J_{6a,5} = 1.5$ Hz, H-6a), 3.89 (ddd, 1H, $J = 10.1$ Hz, 6.1 Hz, 6.1 Hz, octyl OCH₂), 3.74 (dd, 1H, $J_{6b,5} = 2.8$ Hz, H-6b), 3.67 (d, 1H, $J_{2,3} = 10.3$ Hz, H-2), 3.59 (ddd, 1H, $J = 10.3$ Hz, 6.4 Hz, 6.4 Hz, octyl OCH₂), 3.53 (dd, 1H, $J_{3,4} = 8.8$ Hz, H-3), 3.44 (m, 2H, H-4 and H-5), 3.32 (t, 2H, $J = 6.9$ Hz, octyl CH₂N₃), 2.04 (s, 3H, NHCOCH₃), 1.52-1.63 (m, 4H, octyl CH₂), 1.30-1.40 (m, 8H, octyl CH₂); ¹³C NMR (125MHz, D₂O) δ 175.3 (C=O), 102.0 (C-1), 76.7 (C-4 or 5),

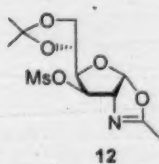
74.7 (C-3), 71.4 (octyl OCH₂), 70.8 (C-4 or 5), 61.7 (C-6), 56.5 (C-2), 52.1 (octyl CH₂N₃), 29.4 (octyl CH₂), 29.2 (octyl CH₂), 29.1 (octyl CH₂), 28.8 (octyl CH₂), 26.8 (octyl CH₂), 25.8 (octyl CH₂), 23.1 (acetyl CH₃); ESI HRMS *m/z* calcd for C₁₆H₃₀N₄O₆Na (M+Na⁺): 397.2058. Found: 397.2058.



2-(Trimethylsilyl)ethyl 2-acetamido-2-deoxy- β -D-glucopyranoside

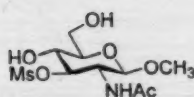
The title compound (196 mg) was prepared from oxazoline **5** and 2-(trimethylsilyl)-ethanol according to the general procedure A and purified by chromatography. Yield 61%. $[\alpha]_D -23.4$ (*c* 0.9, CH₃OH); ¹H NMR (500 MHz, D₂O): δ 4.70 (d, 1H, *J*_{1,2} = 8.5 Hz, H-1), 4.05 (ddd, 1H, *J* = 10.3 Hz, 10.3 Hz, 4.9 Hz, OCH_aH_b), 3.91 (dd, 1H, *J*_{6a,6b} = 12.5 Hz, *J*_{6a,5} = 1.8 Hz, H-6a), 3.75 (m, 1H, H-6b), 3.65-3.73 (m, 2H, OCH_aH_b and H-2), 3.52 (m, 1H, H-3), 3.42-3.48 (m, 2H, H-4 and H-5), 2.03 (s, 3H, NHCOCH₃), 0.98 (ddd, 1H, *J* = 13.8 Hz, 10.6 Hz, 7.3 Hz, OCH_aH_bCH_cH_dSi), 0.88 (ddd, 1H, *J* = 13.8 Hz, 9.8 Hz, 4.9 Hz, OCH_aH_bCH_cH_dSi), 0.3 (s, 9H, SiMe₃); ¹³C NMR (125 MHz, D₂O): δ 175.3, 101.2, 76.8, 75.0, 70.8, 69.1, 61.7, 56.5, 23.1, 18.0, -1.5; ESI HRMS *m/z* calcd. for C₁₃H₂₇NO₆SiNa (M+Na⁺): 344.1500. Found 344.1498.

Chapter Three - Part I
Synthesis of Unusual Amino Sugars



2-Methyl-(3-*O*-methanesulfonyl-1,2-dideoxy-5,6-*O*-isopropylidene- α -D-glucofurano)-[2,1-d]-2-oxazoline (12)

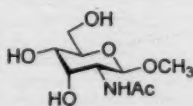
Mesyl chloride (12 mL, 140 mmol) was added to a solution of oxazoline **5** (17 g, 70 mmol) in dry dichloromethane (240 mL) and Et₃N (25 mL) at 0 °C. The mixture was stirred at 0 °C for 2 h and then quenched by adding water. The resulting mixture was extracted several times with dichloromethane. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄. The solvent was evaporated to afford a yellow oil residue that could be directly used in the next glycosylation step without chromatography. A small amount of the mixture was purified by silica-gel chromatography (1:1 toluene-EtOAc) to give the desired product **12** as a white solid. R_f = 0.49 (1:3 toluene-EtOAc); $[\alpha]_D$ +49.6 (c 0.73, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 5.03 (d, 1H, $J_{1,2}$ = 5.0 Hz, H-1), 5.10 (d, 1H, $J_{3,4}$ = 2.9 Hz, H-3), 4.80 (dd, 1H, $J_{2,3}$ = 1.6 Hz, H-2), 4.25 (ddd, 1H, $J_{5,6a}$ = 6.0 Hz, $J_{5,6b}$ = 4.2 Hz, H-5), 4.15 (dd, 1H, $J_{6a,6b}$ = 9.0 Hz, H-6a), 4.03 (dd, 1H, H-6b), 3.85 (dd, 1H, $J_{4,5}$ = 8.5 Hz, H-4), 3.11 (s, 3H, SCH₃), 2.08 (s, 3H, N=CCH₃), 1.42, 1.32 (s, 6H, C(CH₃)₂); ¹³C NMR (125 MHz, CDCl₃) δ 109.7, 106.9, 82.4, 80.4, 72.1, 67.3, 38.0, 26.9, 25.1, 14.1; ESI HRMS m/z Calcd for C₁₂H₂₀NO₇SN⁺ (M+H⁺): 322.0955. Found 322.0956. Anal. Calcd for C₁₂H₁₉NO₇S: C 44.85, H 5.96, N 4.36. Found: C 45.24, H 5.96, N 4.60.



13

Methyl 2-acetamido-2-deoxy-3-(*O*-methanesulfonyl)-β-D-glucopyranoside (13)

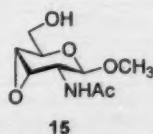
The above residue **12** was dissolved in dry methanol (250 mL) and then *p*-TsOH (1.42 g, 7.5 mmol) was added. The mixture was stirred at room temperature for 18 h and then quenched by addition of Amberlite IRA-400 basic resin (OH⁻). After filtration, the solvent was evaporated and the residue was purified by silica gel chromatography (15:1 CH₂Cl₂-CH₃OH) to afford the desired product **13** as a pale yellow oil residue (15.3 g, 70% over two steps). R_f = 0.26 (10:1 CH₂Cl₂-CH₃OH); $[\alpha]_D$ -78.9 (c 0.92, CH₃OH); ¹H NMR (500 MHz, CD₃OD) δ 8.12 (d, 1H, $J_{NH,H-2}$ = 9.2 Hz, NH), 4.57 (dd, 1H, $J_{3,4}$ = 9.1 Hz, H-3), 4.47 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1), 3.88 (dd, 1H, $J_{6a,6b}$ = 12.0 Hz, H-6a), 3.77 (dd, 1H, $J_{2,3}$ = 10.3 Hz, H-2), 3.72 (dd, 1H, H-6b), 3.57 (dd, 1H, $J_{4,5}$ = 9.6 Hz, H-4), 3.47 (s, 3H, SCH₃), 3.35 (ddd, 1H, $J_{5,6b}$ = 5.4 Hz, $J_{5,6a}$ = 2.6 Hz, H-5), 3.11 (s, 3H, OCH₃), 1.95 (s, 3H, NHCOCH₃); ¹³C NMR (125 MHz, CD₃OD) δ 173.7, 102.8, 85.7, 77.6, 69.9, 62.3, 75.2, 55.7, 39.1, 23.0; ESI HRMS m/z Calcd for C₁₀H₁₉NO₈SN⁺: 336.0723. Found 336.0721. Anal. Calcd for C₁₀H₁₉NO₈S: C 38.33, H 6.11, N 4.47. Found: C 37.79, H 6.43, N 4.14.



14

Methyl 2-acetamido-2-deoxy-β-D-allopyranoside (14)

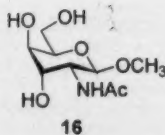
The above compound **13** (15.2 g, 48 mmol) was dissolved in a mixture of methyl cellosolve (2-methoxyethanol) and sat. NaHCO_3 (250 mL, 9:1, v/v). To the above solution NaOAc (20 g) was added and resulting mixture was refluxed for 18 h. After concentration and filtration, the filtrate was concentrated and applied to a silica gel column (15% CH_3OH in CH_2Cl_2) to afford the desired product **14** as a white solid (10.1 g, 89%). $R_f = 0.40$ (5:1 CH_2Cl_2 - CH_3OH); $[\alpha]_D -99.7$ (c 0.77, CH_3OH); ^1H NMR (500 MHz, CD_3OD) δ 4.59 (d, 1H, $J_{1,2} = 8.6$ Hz, H-1), 3.96 (dd, 1H, $J_{3,4} = 2.9$ Hz, H-3), 3.85 (dd, 1H, $J_{6a,6b} = 11.5$ Hz, H-6a), 3.76 (dd, 1H, $J_{2,3} = 2.8$ Hz, H-2), 3.72 (ddd, 1H, $J_{5,6b} = 5.6$ Hz, $J_{5,6a} = 2.2$ Hz, H-5), 3.67 (dd, 1H, H-6b), 3.52 (dd, 1H, $J_{4,5} = 9.6$ Hz, H-4), 3.45 (s, 3H, OCH_3), 1.97 (s, 3H, NHCOCH_3); ^{13}C NMR (125 MHz, CD_3OD) δ 173.0, 101.2, 75.6, 71.4, 68.7, 63.2, 56.9, 54.8, 22.7; ESI HRMS m/z Calcd for $\text{C}_9\text{H}_{17}\text{NO}_6\text{Na}$ ($\text{M}+\text{Na}^+$): 258.0948. Found 258.0950. Anal. Calcd for $\text{C}_9\text{H}_{17}\text{NO}_6$: C 45.95, H 7.28, N 5.95. Found: C 45.72, H 7.35, N 5.81.



Methyl 2-acetamido-3,4-anhydro-2-deoxy- β -D-allopyranoside (**15**)

Compound **13** (11.5 g, 3.7 mmol) was dissolved in dry methanol (90 mL) and then 2 M sodium methoxide in methanol (30 mL) was added. The mixture was stirred at room temperature for 18 h and a white precipitate was formed in the solution. Next the reaction was quenched by the neutralization to pH 7 with acidic resin (Amberlite IR-120). The solution was concentrated to afford a crude product as a solid in quantitative yield. The product could be used directly for next step. A small amount of crude product was further

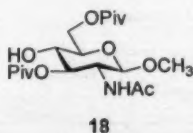
purified by silica gel chromatography using 5% methanol in dichloromethane as eluent to give the desired product **15** as a white solid. $R_f = 0.38$ (10:1 CH_2Cl_2 - CH_3OH); $[\alpha]_D -183.5$ (c 0.93, CH_3OH); ^1H NMR (600 MHz, CD_3OD) δ 4.31 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1), 4.13 (dd, 1H, $J_{2,3} = 2.0$ Hz, H-2), 3.92 (dd, 1H, $J_{3,4} = J_{4,5} = 5.4$ Hz, H-4), 3.78 (dd, 1H, $J_{6a,6b} = 11.5$ Hz, $J_{5,6a} = 5.1$ Hz, H-6a), 3.75 (dd, 1H, $J_{5,6b} = 5.6$ Hz, H-6b), 3.38-3.42 (m, 2H, H-3 and H-5), 3.39 (s, 3H, OCH_3), 1.98 (s, 3H, NHCOCH_3); ^{13}C NMR (125 MHz, CD_3OD) δ 173.5, 100.8, 76.2, 63.5, 57.0, 56.7, 56.0, 51.2, 22.5; ESI HRMS m/z Calcd for $\text{C}_9\text{H}_{15}\text{NO}_5\text{Na}$ ($\text{M}+\text{Na}^+$): 240.0842. Found 240.0842. Anal. Calcd for $\text{C}_9\text{H}_{15}\text{NO}_5$: C 49.76, H 6.96, N 6.45. Found: C 49.65, H 7.01, N 6.26.



Methyl 2-acetamido-2-deoxy- β -D-gulopyranoside (**16**)

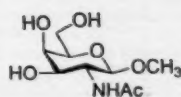
Crude epoxide **15** (4 g, 18 mmol) was dissolved in a mixture of acetone and water (200 mL, 1:1, v/v) and an acidic resin (Amberlite IR-120, 35 g) was added. The mixture was stirred at room temperature until the starting material was almost consumed or stirred at 70 $^\circ\text{C}$ for 2-3 h. After filtration, the filtrate was concentrated and chromatographed on silica gel using an eluent of 10% methanol in dichloromethane to yield the target compound **16** as a white solid (2.7 g, 64%). $R_f = 0.25$ (5:1 CH_2Cl_2 - CH_3OH); $[\alpha]_D -108.3$ (c 0.66, CH_3OH); ^1H NMR (600 MHz, CD_3OD) δ 4.57 (d, 1H, $J_{1,2} = 8.8$ Hz, H-1), 4.10 (dd, 1H, $J_{2,3} = 3.2$ Hz, H-2), 3.92 (ddd, 1H, $J_{5,6a} = 6.7$ Hz, $J_{5,6b} = 5.3$ Hz, H-5), 3.88 (dd, 1H, $J_{3,4} = 3.5$ Hz, H-3), 3.75 (dd, 1H, $J_{6a,6b} = 11.4$ Hz, H-6a), 3.71 (dd, 1H, H-6b), 3.63 (dd, 1H, $J_{4,5} = 1.4$ Hz, H-4), 3.45 (s, 3H, OCH_3), 1.96 (s, 3H, NHCOCH_3); ^{13}C NMR (125

MHz, CD₃OD) δ 173.2, 101.8, 75.1, 71.9, 70.6, 62.8, 56.7, 49.5, 22.8; ESI HRMS m/z Calcd for C₉H₁₇NO₆Na (M+Na⁺): 258.0948. Found 258.0950. Anal. Calcd for C₉H₁₇NO₆: C 45.95, H 7.28, N 5.95. Found: C 45.45, H 7.53, N 5.62.



Methyl 2-acetamido-2-deoxy-3,6-di-O-pivoyl- β -D-glucopyranoside (18)

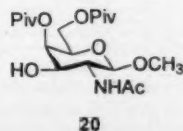
Pivaloyl chloride (13.6 mL, 112 mmol) was added dropwise to a solution of methyl 2-acetamido-2-deoxy- β -D-glucopyranoside **10** (9.4 g, 40 mmol) in a mixture of dry dichloromethane (70 mL) and dry pyridine (170 mL) at 0 °C. The mixture was stirred at 0 °C for 3 h (after 30 min the solution became cloudy). Next the mixture was diluted with dichloromethane (300 mL), washed with sat. NaHCO₃, water, brine and dried over anhydrous Na₂SO₄. After concentration the residue was then purified by silica gel chromatography with hexane and ethyl acetate (1:1) to afford **18** as a white foam (15.2 g, 94%); R_f = 0.20 (1:1 hexane-EtOAc); $[\alpha]_D$ -45.2 (c 0.69, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 5.65 (d, 1H, $J_{NH,2}$ = 9.3 Hz, NH), 5.04 (dd, 1H, $J_{3,4}$ = 8.5 Hz, H-3), 4.42 (dd, 1H, $J_{6a,6b}$ = 12.1 Hz, H-6a), 4.40 (d, 1H, $J_{1,2}$ = 8.2 Hz, H-1), 4.37 (dd, 1H, H-6b), 3.98 (ddd, 1H, $J_{2,3}$ = 10.6 Hz, H-2), 3.55 (ddd, 1H, $J_{5,6a} = J_{5,6b} = 2.4$ Hz, H-5), 3.51 (ddd, 1H, $J_{4,5} = 9.7$ Hz, $J_{4,OH} = 5.1$ Hz, H-4), 3.47 (s, 3H, OCH₃), 3.01 (d, 1H, OH at C-4), 1.94 (s, 3H, NHCOCH₃), 1.24 (s, 9H, C(CH₃)₃), 1.21 (s, 9H, C(CH₃)₃); ¹³C NMR (125 MHz, CDCl₃) δ 179.8, 179.2, 170.0, 102.0, 74.9, 74.3, 69.4, 63.1, 56.4, 53.7, 39.0, 39.0, 27.2, 27.0, 23.3; ESI HRMS m/z Calcd for C₁₉H₃₃NO₈Na (M+Na⁺): 426.2098. Found 426.2102.



19

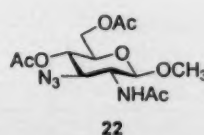
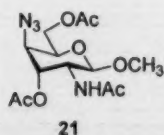
Methyl 2-acetamido-2-deoxy- β -D-galactopyranoside (19)

Trifluoromethanesulfonic anhydride (2.9 mL, 17 mmol) was added dropwise to a solution of compound **18** (6 g, 14.9 mmol) in dry dichloromethane (75 mL) and dry pyridine (7 mL) at $-15\text{ }^{\circ}\text{C}$ under an atmosphere of argon. The mixture was then stirred for several h at this temperature until all starting material had been consumed (monitored by TLC) before warming to RT. Next water (6 mL) was added and the resulting mixture was refluxed for 5-6 h. Then the mixture was diluted with dichloromethane (100 mL), washed with sat. NaHCO_3 , water, brine and dried over anhydrous Na_2SO_4 . After concentration, the residue was dissolved in dry methanol (80 mL) and treated with 2M sodium methoxide in methanol (5 mL) for 18 h at RT. After deionization with Amberlite IR-120 acidic resin, filtration and concentration, the residue was crystallised from 2-propanol to afford the target compound **19** as a white solid (3.13 g, 89%). $R_f = 0.19$ (5:1 CH_2Cl_2 - CH_3OH); $[\alpha]_D -10.8$ (c 0.63, CH_3OH); ^1H NMR (600 MHz, CD_3OD) δ 4.28 (d, 1H, $J_{1,2} = 8.5$ Hz, H-1), 3.90 (dd, 1H, $J_{2,3} = 10.8$ Hz, H-2), 3.82 (dd, 1H, $J_{4,5} = 0.6$ Hz, H-4), 3.77 (dd, 1H, $J_{6a,6b} = 11.4$ Hz, H-6a), 3.73 (dd, 1H, H-6b), 3.56 (dd, 1H, $J_{3,4} = 3.3$ Hz, H-3), 3.48 (ddd, 1H, $J_{5,6a} = 6.7$ Hz, $J_{5,6b} = 5.4$ Hz, H-5), 1.97 (s, 3H, NHCOCH_3); ^{13}C NMR (125 MHz, CD_3OD) δ 174.2, 103.9, 76.7, 73.5, 69.7, 62.5, 56.9, 54.2, 23.0; ESI HRMS m/z Calcd for $\text{C}_9\text{H}_{17}\text{NO}_6\text{Na}$ ($\text{M}+\text{Na}^+$): 258.0948. Found 258.0946. Anal. Calcd for $\text{C}_9\text{H}_{17}\text{NO}_6$: C 45.95, H 7.28, N 5.95. Found: C 45.59, H 7.25, N 5.93.



Methyl 2-acetamido-2-deoxy-4,6-di-O-pivoyl- β -D-galactopyranoside (20)

Compound **20** was prepared from **18** following the above procedure of compound **19** without transesterification step. A small amount of crude **20** was purified as a white solid by silica gel chromatography using 3% dichloromethane in methanol as eluent. $R_f = 0.22$ (1:1 hexane-EtOAc); $[\alpha]_D -23.4$ (c 1.07, CHCl_3); ^1H NMR (500 MHz, CDCl_3) δ 5.84 (bs, 1H, NH), 5.30 (d, 1H, $J_{4,5} = 3.7$ Hz, H-4), 4.42 (d, 1H, $J_{1,2} = 8.2$ Hz, H-1), 4.17 (dd, 1H, $J_{6a,6b} = 11.3$ Hz, $J_{6a,5} = 7.3$ Hz, H-6a), 4.09 (dd, 1H, $J_{6b,5} = 6.1$ Hz, H-6b), 3.99 (dd, 1H, $J_{3,4} = 3.5$ Hz, H-3), 3.89 (dd, 1H, $J_{2,3} = 10.4$ Hz, H-2), 3.69 (m, 1H, H-5), 3.51 (s, 3H, OCH_3), 2.06 (s, 3H, NHCOCH_3), 1.26 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.19 (s, 9H, $\text{C}(\text{CH}_3)_3$); ^{13}C NMR (125 MHz, CDCl_3) δ 178.0, 177.8, 173.1, 101.2, 72.0, 72.0, 72.0, 71.4, 68.4, 61.86, 55.7, 55.7, 39.2, 38.7, 27.2, 27.0, 23.4; ESI HRMS m/z Calcd for $\text{C}_{19}\text{H}_{33}\text{NO}_8\text{Na}$ ($\text{M}+\text{Na}^+$): 426.2098. Found 426.2095.



Methyl 2-acetamido-3,6-di-O-acetyl-4-azido-2,4-dideoxy- β -D-gulopyranoside (21)

Methyl 2-acetamido-3,6-di-O-acetyl-4-azido-2,4-dideoxy- β -D-glucopyranoside (22)

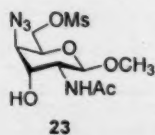
Epoxide **15** (1.09 g, 5 mmol) was dissolved in dry DMF and then NaN_3 (1 g) and NH_4Cl (1 g) were added. The resulting mixture was stirred at 90 $^\circ\text{C}$ for 18h. After the evaporation of the solvent and washing with methanol, the solid residue was removed by

filtration. The filtrates were combined and evaporated under reduced pressure to afford a colourless oil mixture. The mixture was dissolved in dry pyridine (50 mL) and acetic anhydride (5 mL) was then added at 0 °C. The mixture was stirred from 0 °C to room temperature for 18 h. After the removal of solvent, the resulting mixture was separated by silica gel chromatography using a gradient eluent of toluene and ethyl acetate (1:1 to 1:2) to provide two pure compounds **21** (1.32 g, 77%) and **22** (0.3 g, 17%) both as white solids.

Compound 21: $R_f = 0.27$ (1:6 toluene-EtOAc); $[\alpha]_D -84.9$ (c 0.94, CHCl_3); ^1H NMR (500 MHz, CDCl_3) δ 5.44 (d, 1H, $J_{\text{NH,H-2}} = 8.2$ Hz, NH), 5.33 (dd, 1H, $J_{3,4} = 3.6$ Hz, H-3), 4.54 (d, 1H, $J_{1,2} = 8.4$ Hz, H-1), 4.38 (ddd, 1H, $J_{2,3} = 3.3$ Hz, H-2), 4.34 (dd, 1H, $J_{6a,6b} = 11.5$ Hz, H-6a), 4.20 (dd, 1H, H-6b), 4.06 (ddd, 1H, $J_{5,6a} = J_{5,6b} = 6.6$ Hz, H-5), 3.74 (dd, 1H, $J_{4,5} = 2.0$ Hz, H-4), 3.47 (s, 3H, OCH_3), 2.16 (s, 3H, COCH_3), 2.10 (s, 3H, COCH_3), 2.01 (s, 3H, NHCOCH_3); ^{13}C NMR (125 MHz, CDCl_3) δ 170.5, 169.7, 169.3, 100.0, 70.9, 70.2, 62.7, 58.4, 55.9, 47.9, 23.3, 20.9, 20.8; ESI HRMS m/z Calcd for $\text{C}_{13}\text{H}_{20}\text{N}_4\text{O}_7\text{Na}$ ($\text{M}+\text{Na}^+$): 367.1224. Found 367.1223. Anal. Calcd for $\text{C}_{13}\text{H}_{20}\text{N}_4\text{O}_7$: C 45.35, H 5.85, N 16.27. Found: C 44.93, H 5.86, N 16.47.

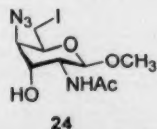
Compound 22: $R_f = 0.41$ (1:6 toluene-EtOAc); $[\alpha]_D +62.2$ (c 0.36, CHCl_3); ^1H NMR (500 MHz, CDCl_3) δ 5.83 (d, 1H, $J_{\text{NH,H-2}} = 7.1$ Hz, NH), 5.00 (d, 1H, $J_{1,2} = 8.2$ Hz, H-1), 4.90 (dd, 1H, $J_{4,5} = 9.9$ Hz, H-4), 4.51 (dd, 1H, $J_{3,4} = 10.0$ Hz, H-3), 4.27 (dd, 1H, $J_{6a,6b} = 12.3$ Hz, H-6a), 4.10 (dd, 1H, H-6b), 3.72 (ddd, 1H, $J_{5,6a} = 4.9$ Hz, $J_{5,6b} = 2.6$ Hz, H-5), 3.51 (s, 3H, OCH_3), 3.08 (ddd, $J_{2,3} = 11.0$ Hz, H-2), 2.12 (s, 3H, COCH_3), 2.08 (s, 3H, COCH_3), 2.04 (s, 3H, NHCOCH_3); ^{13}C NMR (125 MHz, CDCl_3) δ 171.1, 170.7, 169.5, 100.0, 72.4, 69.6, 62.1, 61.7, 57.6, 57.1, 23.6, 20.7, 20.7; ESI HRMS m/z Calcd for

$C_{13}H_{20}N_4O_7Na$ ($M+Na^+$): 367.1224. Found 367.1221. Anal. Calcd for $C_{13}H_{20}N_4O_7$: C 45.35, H 5.85, N 16.27. Found: C 45.34, H 5.91, N 15.93.



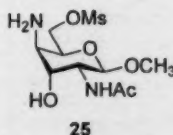
Methyl 2-acetamido-4-azido-2,4-dideoxy-6-O-methanesulfonyl- β -D-gulopyranoside
(23)

Compound **21** (0.99 g, 2.6 mmol) was dissolved in anhydrous methanol (50 mL) and then 2 M sodium methoxide in methanol (0.5 mL) was added. The mixture was stirred at room temperature for 18 h and then quenched by addition of acidic resin (Amberlite IR-120). Filtration and solvent removal gave a solid residue, which was redissolved dry pyridine (15 mL) and followed by addition of 1 equiv of mesyl chloride (0.2 mL, 2.6 mmol) at -20°C . The mixture was stirred at -20°C for 18 h. After concentration, the residue was purified by silica gel chromatography using 5% methanol in dichloromethane as eluent to yield the desired product **23** as a white solid (695 mg, 79%). $R_f = 0.43$ (10:1 CH_2Cl_2 - CH_3OH); $[\alpha]_D -123$ (c 0.88, CH_3OH); ^1H NMR (600 MHz, CD_3OD) δ 4.63 (d, 1H, $J_{1,2} = 8.7$ Hz, H-1), 4.34-4.38 (m, 2H, H-6a and H-6b), 4.30 (d, 1H, $J_{5,6a} = J_{5,6b} = 6.3$ Hz, H-5), 4.11 (dd, 1H, $J_{3,4} = 3.3$ Hz, H-3), 4.02 (dd, 1H, $J_{2,3} = 3.1$ Hz, H-2), 3.70 (dd, 1H, $J_{4,5} = 1.8$ Hz, H-4), 3.44 (s, 3H, SCH_3), 3.12 (s, 3H, OCH_3), 1.97 (s, 3H, NHCOCH_3); ^{13}C NMR (125 MHz, CD_3OD) δ 173.2, 101.5, 71.2, 70.0, 69.4, 62.9, 56.9, 51.3, 37.3, 22.7; ESI HRMS m/z Calcd for $C_{10}H_{18}N_4O_7\text{SNa}$ ($M+Na^+$): 361.0788. Found 361.0788. Anal. Calcd for $C_{10}H_{18}N_4O_7\text{S}$: C 35.50, H 5.36, N 16.56. Found: C 35.15, H 5.27, N 16.00.



Methyl 2-acetamido-4-azido-2,4,6-trideoxy-6-iodo-β-D-gulopyranoside (24)

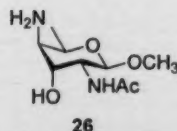
To a solution of compound **23** (305 mg, 0.9 mmol) in dry acetone (10 mL) was added NaI (1 g). The mixture was stirred at 90 °C for 18 h. The mixture was concentrated and purified by silica gel chromatography using 5% methanol in dichloromethane as eluent to give the desired product **24** as a white solid (260 mg, 78%). $R_f = 0.43$ (10:1 CH₂Cl₂-CH₃OH); ¹H NMR (400 MHz, CD₃OD) δ 4.57 (d, 1H, $J_{1,2} = 8.7$ Hz, H-1), 4.15 (ddd, 1H, $J_{5,6} = 7.9$ Hz, 6.3 Hz, H-5), 4.08 (dd, 1H, $J_{3,4} = 3.4$ Hz, H-3), 3.96 (dd, 1H, $J_{2,3} = 3.2$ Hz, H-2), 3.85 (dd, 1H, $J_{4,5} = 1.7$ Hz, H-4), 3.46 (s, 3H, OCH₃), 3.30-3.36 (m, 2H, H-6a and H-6b), 1.98 (s, 3H, NHCOCH₃); ESI HRMS m/z Calcd for C₉H₁₅N₄O₄INa (M+Na⁺): 393.0030. Found 393.0030.



Methyl 2-acetamido-4-amino-2,4-dideoxy-6-(O-methanesulfonyl)-β-D-gulopyranoside (25)

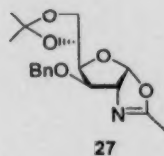
To a solution of compound **23** (20 mg, 0.06 mmol) in ethanol (5 mL) were successively added NaBH₄ (15 mg) and 0.16 M NiCl₂ (0.2 mL). The mixture was then stirred at RT for 18 h and then quenched with acetic acid (1 mL). The mixture was concentrated and purified by silica gel chromatography (100:10:1 CH₂Cl₂-CH₃OH-NH₄OH) to provide the compound **25** as colorless oil (14.5 mg, 77%); $R_f = 0.30$ (5:1 CH₂Cl₂-CH₃OH); $[\alpha]_D -$

72.8 (*c* 0.28, CH₃OH); ¹H NMR (500 MHz, CD₃OD) δ 4.57 (d, 1H, $J_{1,2}$ = 8.7 Hz, H-1), 4.40 (dd, 1H, $J_{6a,6b}$ = 11.1 Hz, H-6a), 4.34 (dd, 1H, H-6b), 4.25 (ddd, 1H, $J_{5,6a}$ = 7.9 Hz, $J_{5,6b}$ = 4.1 Hz, H-5), 4.02 (dd, 1H, $J_{2,3}$ = 3.1 Hz, H-2), 3.84 (dd, 1H, $J_{3,4}$ = 3.3 Hz, H-3), 3.43 (s, 3H, SCH₃), 3.11 (s, 3H, OCH₃), 2.97 (dd, 1H, $J_{4,5}$ = 1.8 Hz, H-4), 1.97 (s, 3H, NHCOCH₃); ¹³C NMR (125 MHz, CD₃OD) δ 173.1, 102.2, 72.9, 72.4, 71.2, 56.9, 54.0, 50.9, 37.3, 22.7; ESI HRMS *m/z* Calcd for C₁₀H₂₀N₂O₇SNa (M+Na⁺): 335.1072. Found 335.1069.



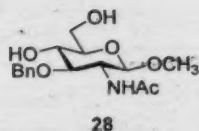
Methyl 2-acetamido-4-amino-2,4,6-trideoxy- β -D-gulopyranoside (26)

To a solution of compound **23** (110 mg, 0.32 mmol) in ethanol (10 mL) were added NaBH₄ (100 mg) and 0.16 M NiCl₂ in ethanol (0.2 mL). The mixture was then stirred at 50–60 °C for 18 h and then quenched by acetic acid (1 mL). The mixture was concentrated for silica gel chromatography (100:10:1 CH₂Cl₂-CH₃OH-NH₄OH) to afford the desired product **26** as colorless oil residue (52 mg, 74%). R_f = 0.17 (5:1 CH₂Cl₂:CH₃OH); $[\alpha]_D$ –93.8 (*c* 0.88, CH₃OH); ¹H NMR (600 MHz, CD₃OD) δ 4.52 (d, 1H, $J_{1,2}$ = 8.8 Hz, H-1), 4.13 (qd, $J_{5,6}$ = 6.6 Hz, H-5), 3.93 (dd, 1H, $J_{2,3}$ = 3.2 Hz, H-2), 3.86 (dd, 1H, $J_{3,4}$ = 3.3 Hz, H-3), 2.65 (dd, 1H, $J_{4,5}$ = 1.8 Hz, H-4), 1.96 (s, 3H, NHCOCH₃), 1.30 (d, 3H, H-6 CH₃); ¹³C NMR (125 MHz, CD₃OD) δ 173.1, 102.1, 72.9, 69.6, 56.7, 56.6, 50.9, 22.7, 16.7; ESI HRMS *m/z* Calcd for C₉H₁₈N₂O₄Na (M+Na⁺): 241.1159. Found: 241.1156.



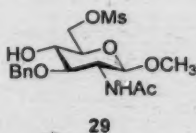
2-Methyl-(3-*O*-benzyl-1,2-dideoxy-5,6-*O*-isopropylidene- α -D-glucofurano)-[2,1-d]-2-oxazoline (27)

Oxazoline **5** (6 g, 24.7 mmol) was dissolved in dry DMF (150 mL) and then a 60% dispersion of sodium hydride in mineral oil (5 g) was added. After stirring for 30 min at RT, benzyl bromide (5 mL) was added dropwise at 0 °C. The mixture was then stirred for 18 h from 0 °C to RT. The reaction was quenched by addition of methanol (30 mL) and concentrated followed by addition of water (100 mL). The mixture was extracted several times with dichloromethane and the combined extracts were dried over anhydrous Na₂SO₄. After concentration, the residue was passed through a short silica pad washed with dichloromethane before further purification by silica gel chromatography (1:1 hexane-ethyl acetate containing 1% Et₃N) to afford compound **27** as a pale-yellow oil (5.56 g, 68%). R_f = 0.47 (1:3 hexane-EtOAc); $[\alpha]_D$ -27.6 (c 0.92, CHCl₃); {lit: $[\alpha]_D$ -36 (c 1.2, CHCl₃)}; ¹⁴H NMR (600 MHz, CDCl₃) δ 7.34-7.38 (m, 4H, ArH), 7.28-7.32 (m, 1H, ArH), 6.13 (d, 1H, $J_{1,2}$ = 5.1 Hz, H-1), 4.73 (d, 1H, J = 11.8 Hz, PhCH₂), 4.66 (d, 1H, J = 11.8 Hz, PhCH₂), 4.53 (dd, 1H, $J_{2,3}$ = 1.2 Hz, H-2), 4.38 (dd, 1H, $J_{5,6a}$ = $J_{5,6b}$ = 5.9 Hz, H-5), 4.10 (dd, 1H, $J_{6a,6b}$ = 8.6 Hz, H-6a), 4.20 (dd, 1H, H-6b), 4.11 (d, 1H, $J_{3,4}$ = 3.2 Hz, H-3), 3.85 (dd, 1H, $J_{4,5}$ = 7.1 Hz, H-4), 1.42, 1.37 (s, 6H, C(CH₃)₂); ¹³C NMR (125 MHz, CDCl₃) δ 167.0, 137.6, 128.4, 127.8, 127.8, 127.8, 127.8, 127.7, 109.0, 107.1, 81.6, 75.4, 72.6, 72.2, 67.0, 26.7, 25.3, 14.2; ESI HRMS m/z Calcd for C₁₈H₂₄NO₅Na (M+Na⁺): 334.1649. Found 334.1646.



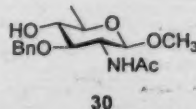
Methyl 2-acetamido-3-O-benzyl-2-deoxy- β -D-glucopyranoside (28)

Compound **27** (5.48 g, 16.4 mmol) was dissolved in dry methanol (150 mL) and camphor-10-sulfonic acid (1.04 g, 4.5 mmol) was added. The mixture was stirred at RT for 36 h, and then the reaction was quenched by Amberlite IRA-400 basic resin (OH⁻). After filtration, the filtrate was concentrated for silica gel chromatography (5% methanol in dichloromethane) to afford the desired compound **28** as a white solid (3.73 g, 70%): R_f = 0.29 (10:1 CH₂Cl₂-CH₃OH); $[\alpha]_D$ -26.6 (c 0.74, CH₃OH); ¹H NMR (500 MHz, CD₃OD) δ 7.20-7.32 (m, 5H, ArH), 4.86 (d, 1H, J = 11.4 Hz, PhCH₂), 4.64 (d, 1H, J = 11.4 Hz, PhCH₂), 4.34 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1), 3.89 (dd, 1H, $J_{6a,6b}$ = 11.9 Hz, H-6a), 3.74 (dd, 1H, $J_{2,3}$ = 9.8 Hz, H-2), 3.70 (dd, 1H, H-6b), 3.47-3.52 (m, 2H, H-4 and H-3), 3.28 (dd, 1H, $J_{5,6b}$ = 6.0 Hz, $J_{5,6a}$ = 2.4 Hz, H-5), 3.45 (s, 3H, OCH₃), 1.86 (s, 3H, NHCOCH₃); ¹³C NMR (125 MHz, CD₃OD) δ 173.3, 140.3, 129.2, 128.8, 128.5, 103.5, 84.2, 78.0, 75.6, 72.1, 62.7, 57.0, 56.2, 23.0; ESI HRMS m/z Calcd for C₁₆H₂₃NO₆Na (M+Na⁺): 348.1417. Found 348.1411. Anal. Calcd for C₁₆H₂₃NO₆: C 59.06, H 7.13, N 4.31. Found: C 58.85, H 7.14, N 4.36.



Methyl 2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-methanesulfonyl- β -D-glucopyranoside (29)

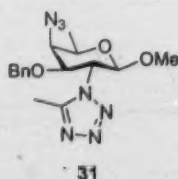
Compound **28** (1.2 g, 3.7 mmol) was dissolved in dry pyridine (30 mL) first and then cooled to -15°C . To the mixture mesyl chloride (1.05 eq, 0.31 mL, 4 mmol) was added dropwise. The mixture was stirred at this temperature for 3-4 h until all starting material have been consumed. The reaction was quenched by addition of methanol (1 mL) and the solvent was evaporated under reduced pressure. The resulting residue was purified by silica gel chromatography using 2.5% methanol in dichloromethane as eluent to afford the desired compound **29** as a white solid (1.32 g, 88%). $R_f = 0.53$ (10:1 CH_2Cl_2 - CH_3OH); $[\alpha]_D +1.8$ (c 2.09, CH_3OH); ^1H NMR (500 MHz, CD_3OD) δ 7.22-7.34 (m, 5H, ArH), 4.87 (d, 1H, $J = 11.4$ Hz, PhCH_2), 4.65 (d, 1H, $J = 11.4$ Hz, PhCH_2), 4.55 (dd, 1H, $J_{6a,6b} = 11.2$ Hz, H-6a), 4.41 (dd, 1H, $J_{6b,5} = 5.0$ Hz, H-6b), 4.39 (d, 1H, $J_{1,2} = 8.4$ Hz, H-1), 3.73 (dd, 1H, $J_{2,3} = 8.7$ Hz, H-2), 3.48-3.55 (m, 3H, H-3, H-4 and H-5), 3.44 (s, 3H, SCH_3), 3.11 (s, 3H, OCH_3), 1.86 (s, 3H, NHCOCH_3); ^{13}C NMR (125 MHz, CD_3OD) δ 173.4, 140.1, 129.3, 128.8, 128.6, 103.4, 83.8, 75.8, 75.3, 71.5, 70.4, 57.1, 56.1, 37.5, 23.0; ESI HRMS m/z Calcd for $\text{C}_{17}\text{H}_{25}\text{NO}_8\text{SNa}$ ($\text{M}+\text{Na}^+$): 426.1193. Found 426.1196. Anal. Calcd for $\text{C}_{17}\text{H}_{25}\text{NO}_8\text{S}$: C 50.61, H 6.25, N 3.47. Found: C 50.60, H 6.22, N 3.54.



Methyl 2-acetamido-3-*O*-benzyl-2,6-dideoxy- β -D-glucopyranoside (30)

Compound **29** (1.1 g, 2.7 mmol) was dissolved in dry DMSO (20 mL) and then NaBH_4 (1 g) was added. The mixture was heated at $85-90^{\circ}\text{C}$ for 18 h and the reaction was

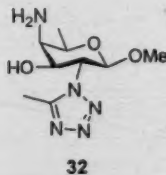
quenched by adding a 5% acetic acid solution (2 mL) at 0 °C until it became a clear solution. Next the solution was extracted several times with ethyl acetate. The combined extracts were evaporated under reduced pressure to yield a residue, which was chromatographed on silica gel using toluene/ethyl acetate (1:2) as eluent providing the title compound **30** as a white solid (0.69 g, 83%). $R_f = 0.28$ (1:10 hexane-EtOAc); $[\alpha]_D - 9.6$ (c 0.75, CHCl_3); ^1H NMR (600 MHz, CDCl_3) δ 7.30-7.40 (m, 5H, ArH), 5.60 (d, 1H, $J_{\text{NH,H-2}} = 7.3$ Hz, NH), 4.76 (d, 1H, $J_{1,2} = 8.1$ Hz, H-1), 4.75 (d, 1H, $J = 12.4$ Hz, PhCH_2), 4.70 (d, 1H, $J = 11.7$ Hz, PhCH_2), 3.99 (dd, 1H, $J_{3,4} = 8.8$ Hz, H-3), 3.48 (s, 3H, OCH_3), 3.42 (qd, 1H, $J_{5,6} = 6.2$ Hz, H-5), 3.30 (ddd, 1H, $J_{2,3} = 10.3$ Hz, H-2), 3.27 (dd, 1H, $J_{4,5} = 9.1$ Hz, H-4), 1.96 (s, 3H, NHCOCH_3), 1.33 (d, 3H, $J = 6.3$ Hz, H-6 CH_3); ^{13}C NMR (125 MHz, CDCl_3) δ 170.6, 138.3, 128.7, 128.1, 128.0, 128.0, 100.5, 80.7, 76.1, 74.1, 71.4, 57.7, 56.7, 23.7, 17.7; ESI HRMS m/z Calcd for $\text{C}_{16}\text{H}_{23}\text{NO}_5\text{Na}$ ($\text{M}+\text{Na}^+$): 332.1468. Found: 332.1465. Anal. Calcd for $\text{C}_{16}\text{H}_{23}\text{NO}_5$: C 62.12, H 7.49, N 4.53. Found: C 61.98, H 7.20, N 4.50.



1-Methyl-5-[methyl 4-azido-3-O-benzyl-2,4,6-trideoxy- β -D-galactopyranosid-2-]-1H-tetrazole (31)

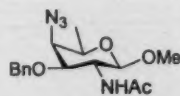
To a solution of compound **30** (430 mg, 1.4 mmol) in dry dichloromethane (30 mL) and dry pyridine (3 mL) was added dropwise trifluoromethanesulfonic anhydride (0.48 mL, 2.8 mmol) at -10 °C under argon. The mixture was stirred at -10 °C for 3 h and then

diluted with dichloromethane (20 mL), washed successively with 1M HCl, sat. NaHCO₃ solution, and water. The organic layer was dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure (< 20 °C bath) to afford a yellow oily residue for the next step. This residue was redissolved in dry DMF (5 mL) and NaN₃ (450 mg, 7 mmol) was added. The mixture was stirred at room temperature for 18 h. After concentration, the residue was dissolved in dichloromethane, washed with water, brine and dried over anhydrous Na₂SO₄. After filtration and solvent removal, the crude residue was purified by silica gel chromatography (10:1 toluene-ethyl acetate) to afford **31** as a white solid (438 mg, 87%): $R_f = 0.37$ (4:1 toluene-EtOAc); $[\alpha]_D -1.8$ (c 1.13, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 7.28-7.32 (m, 3H, ArH), 7.20-7.25 (m, 2H, ArH), 4.70 (d, 1H, $J_{1,2} = 8.1$ Hz, H-1), 4.45 (d, 1H, $J = 11.4$ Hz, PhCH₂), 4.39 (dd, 1H, $J_{3,4} = 3.5$ Hz, H-3), 4.33 (d, 1H, $J = 11.2$ Hz, PhCH₂), 3.78 (qd, 1H, $J_{5,6} = 6.3$ Hz, H-5), 3.76 (dd, 1H, $J_{4,5} = 1.3$ Hz, H-4), 3.34 (s, 3H, OCH₃), 2.54 (s, 3H, tetrazole CH₃), 1.41 (d, 3H, H-6 CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 154.2, 136.1, 128.7, 128.5, 128.1, 101.6, 78.6, 72.8, 69.4, 62.5, 59.5, 57.2, 17.5, 8.9; ESI HRMS m/z Calcd for C₁₆H₂₁N₇O₃Na (M+Na⁺): 382.1298. Found 382.1598. Anal. Calcd for C₁₆H₂₁N₇O₃: C 53.47, H 5.89, N 27.28. Found: C 53.38, H 5.81, N 26.89.



1-Methyl-5-[methyl 4-amino-2,4,6-dideoxy-β-D-galactopyranosid-2-yl]-1H-tetrazole
(32)

To a solution of compound **31** (68 mg, 0.19 mmol) in dry methanol (2 mL) containing acetic acid (0.2 mL), 10% palladium on carbon (100 mg) was added. The mixture was then stirred at room temperature under a hydrogen atmosphere for 18 h. After filtration and concentration, the crude residue was purified by silica gel chromatography using 5% methanol in dichloromethane as eluent to afford **32** as a white solid (42 mg, 91%); R_f = 0.39 (5:1 CH_2Cl_2 - CH_3OH); $[\alpha]_D +12.8$ (c 0.69, CH_3OH); ^1H NMR (400 MHz, CD_3OD) δ 4.78 (d, 1H $J_{1,2}$ = 8.1 Hz, H-1), 4.30-3.36 (m, 2H, J = 10.6 Hz, 8.8 Hz, 2.8 Hz, H-3 and H-2), 3.94 (qt, 1H, $J_{5,6}$ = 6.5 Hz, H-5), 3.34 (s, 3H, OCH_3), 3.02 (dd, 1H, $J_{4,3}$ = 3.4 Hz, $J_{4,5}$ = 1.7 Hz, H-4), 2.57 (s, 3H, tetrazole CH_3), 1.36 (d, 3H, H-6 CH_3); ^{13}C NMR (125 MHz, CD_3OD) δ 155.8 (tet-C), 103.2 (C-1), 71.9, 71.4, 61.9, 57.2, 56.2, 17.1, 8.7 (CH_3 -CN); ESI HRMS m/z Calcd for $\text{C}_9\text{H}_{18}\text{N}_5\text{O}_3\text{Na}$ ($\text{M}+\text{Na}^+$): 244.1404. Found 244.1404.

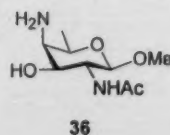


35

Methyl 2-acetamido-4-azido-3-O-benzyl-2,4,6-trideoxy- β -D-galactopyranoside (35)

Compound **30** (300 mg, 0.97 mmol) was dissolved in dry dichloromethane (10 mL) and dry pyridine (1 mL) under argon. After cooling the solution to $-30\text{ }^\circ\text{C}$, trifluoromethanesulfonic anhydride (328 mg, 0.196 mL, 1.16 mmol) was added dropwise over approximately 1 min. The mixture was then stirred at $-30\text{ }^\circ\text{C}$ for 3 h and diluted with dichloromethane (20 mL), washed successively with 1M HCl, sat. NaHCO_3 solution, and water. The organic layer was dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure ($<20\text{ }^\circ\text{C}$) to afford an oily residue for the next step. This residue was redissolved in dry DMF (5 mL) and then NaN_3 (325 mg, 5 mmol) was added. The

mixture was stirred at RT for 18 h. After concentration, the residue was dissolved in dichloromethane, washed with water, brine and dried over anhydrous Na_2SO_4 . After filtration and solvent removal, the residue was purified by silica gel chromatography (1:1 toluene-ethyl acetate) to yield compound **35** as a white solid (213 mg, 66%): $R_f = 0.20$ (1:2 toluene-EtOAc); $[\alpha]_D +44.5$ (c 0.53, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.30-7.40 (m, 5H, ArH), 5.68 (d, 1H, $J_{\text{NH,H-2}} = 7.8$ Hz, NH), 4.91 (d, 1H, $J_{1,2} = 8.4$ Hz, H-1), 4.70 (d, 1H, $J = 11.4$ Hz, PhCH_2), 4.62 (dd, 1H, $J_{3,4} = 3.6$ Hz, H-3), 4.56 (d, 1H, $J = 11.4$ Hz, PhCH_2), 3.72 (dd, 1H, $J_{4,5} = 0.9$ Hz, H-4), 3.68 (t, 1H, $J_{5,6} = 6.5$ Hz, H-5), 3.20 (ddd, 1H, $J_{2,3} = 10.6$ Hz, H-2), 3.47 (s, 3H, OCH_3), 1.94 (s, 3H, NHCOCH_3), 1.33 (d, 3H, H-6 CH_3); ^{13}C NMR (125 MHz, CDCl_3) δ 171.0, 137.5, 128.6, 128.2, 128.2, 99.9, 76.4, 72.6, 68.8, 63.4, 56.7, 55.5, 23.8, 17.6; ESI HRMS m/z Calcd for $\text{C}_{16}\text{H}_{22}\text{N}_4\text{O}_4\text{Na}$ ($\text{M}+\text{Na}^+$): 357.1533. Found: 357.1534. Anal. Calcd for $\text{C}_{16}\text{H}_{22}\text{N}_4\text{O}_4$: C 57.47, H 6.63, N 16.76. Found: C 57.77, H 6.61, N 15.83.



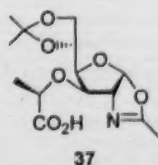
Methyl 2-acetamido-4-amino-2,4,6-trideoxy- β -D-galactopyranoside (**36**)

Compound **35** (150 mg, 0.45 mmol) was dissolved in dry methanol (5 mL) and then 20% palladium hydroxide on carbon (100 mg) was added. The mixture was stirred under a hydrogen atmosphere at RT for 18 h. After filtration, the solution was concentrated under reduce pressure followed by silica gel chromatography using dichloromethane/methanol/ammonium hydroxide (100:10:1) as eluent to afford the target compound **36** as a white solid (91 mg, 93%): $R_f = 0.33$ (100:20:1 $\text{CH}_2\text{Cl}_2\text{-CH}_3\text{OH}$ -

NH₄OH); $[\alpha]_D -10.8$ (*c* 0.91, CH₃OH); lit: $^{[102]}[\alpha]_D -8.7$ (*c* 1.0, CH₃OH); ¹H NMR (600 MHz, CD₃OD) δ 4.43 (d, 1H, $J_{1,2} = 8.7$ Hz, H-1), 4.02-4.07 (m, 2H, $J = 10.7$ Hz, $J = 4.5$ Hz, H-3 and H-5), 3.75 (dd, 1H, $J_{2,3} = 10.9$ Hz, H-2), 3.59 (d, 1H, $J = 4.6$ Hz, H-4), 3.50 (s, 3H, OCH₃), 2.04 (s, 3H, NHCOCH₃), 1.34 (d, 3H, $J_{6,5} = 6.7$ Hz, H-6 CH₃); ¹³C NMR (125 MHz, CD₃OD) δ 176.0, 103.3, 68.8, 68.5, 58.3, 55.7, 52.9, 23.1, 16.5; ESI HRMS *m/z* Calcd for C₉H₁₉N₂O₄ (M+H⁺): 219.1339 Found: 219.1336.

Chapter Three – Part II

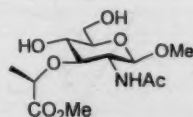
Synthesis of Muramic Acid and Peptide Derivatives



2-methyl-(1,2-dideoxy-5,6-*O*-isopropylidene-3-*O*-[(*R*)-1-carboxyethyl]-α-*D*-glucofuran)-[2,1-*d*]-2-oxazoline (37)

To a solution of oxazoline **5** (1.2 g, 5 mmol) in dry DMF (50 mL) was added a 60% dispersion of NaH in mineral oil (4 g) that has been previously washed with hexane at room temperature. Then the mixture was stirred at 60 °C for 20-30 min. Next a solution of (*S*)-2-bromopropionic acid (1.35 mL, 15 mmol) in dry DMF (5 mL) was added and then the mixture was warmed to 60 °C with stirring for 4-5 h. After the removal of solvent, the residue was dissolved in a minimum amount of methanol and filtered through a short pad of silica gel by washing with 20% methanol in dichloromethane containing 1% NH₄OH. The filtrate was concentrated under reduced pressure to give a crude residue

that was used in the next step without further purification. A small amount of residue was purified by chromatography on silica gel using dichloromethane/methanol/ NH_4OH (100:10:1) as eluent to afford compound **37**: $R_f = 0.22$ (100:5:0.5 dichloromethane-methanol- NH_4OH); ^1H NMR (600 MHz, CDCl_3): δ 6.15 (d, 1H, $J_{1,2} = 5.2$ Hz, H-1), 4.84 (dd, 1H, $J_{2,3} = 1.0$ Hz, H-2), 4.28 (ddd, 1H, $J_{5,6a} = 6.3$ Hz, $J_{5,6b} = 5.9$ Hz, H-5), 4.14 (q, 1H, $J = 6.8$ Hz, $-\text{CH}(\text{CO}_2\text{H}, \text{CH}_3)$), 4.10 (dd, 1H, $J_{6a,6b} = 8.6$ Hz, H-6a), 4.02 (d, 1H, $J_{3,4} = 3.1$ Hz, H-3), 3.95 (dd, 1H, H-6b), 3.71 (dd, 1H, $J_{4,5} = 7.9$ Hz, H-4), 1.98 (s, 3H, $\text{N}=\text{CCH}_3$), 1.38 (s, 3H, $-\text{C}(\text{CH}_3)_2$), 1.37 (d, 3H, $J = 6.8$ Hz, $-\text{CH}_3(\text{H}, \text{CO}_2\text{H})$), 1.33 (s, 3H, $-\text{C}(\text{CH}_3)_2$).

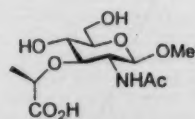


38

Methyl 2-acetamido-2-deoxy-3-*O*-[(*R*)-1-(methoxycarbonyl)-ethyl]- β -D-glucopyranoside (38)

Crude residue **37** was dissolved in dry methanol (80 mL) and then *p*-TsOH (0.95 g, 5 mmol) was added (pH of solution is in the range of 2-3). The mixture was stirred at room temperature overnight and quenched by addition of amberlite IRA-400 basic resin. After filtration and concentration of the filtrate, the resulting residue was purified by silica gel chromatography using 8% methanol in dichloromethane as eluent to afford the desired product **38** as a white solid (1.08 g, 67%): $R_f = 0.32$ (10:1 CH_2Cl_2 - CH_3OH); $[\alpha]_D -4.6$ (c 1.69, CH_3OH); ^1H NMR (600 MHz, CD_3OD) δ 4.52 (q, 1H, $J = 6.8$ Hz, $-\text{CH}(\text{CO}_2\text{CH}_3, \text{CH}_3)$), 4.32 (d, 1H, $J_{1,2} = 8.5$ Hz, H-1), 3.85 (dd, 1H, $J_{6a,6b} = 11.9$ Hz, H-6a), 3.71 (s, 3H, CO_2CH_3), 3.66 (dd, 1H, H-6b), 3.59 (dd, 1H, $J_{2,3} = 10$ Hz, H-2), 3.44 (dd, 1H,

$J_{3,4} = 8.7$ Hz, H-3), 3.44 (s, 3H, OCH₃), 3.40 (dd, 1H, $J_{4,5} = 8.7$ Hz, H-4), 3.23 (ddd, 1H, $J_{5,6b} = 5.8$ Hz, $J_{5,6a} = 2.3$ Hz, H-5), 1.97 (s, 3H, NHCOCH₃), 1.35 (d, 3H, $J = 6.8$ Hz, -CH₃(H, CO₂CH₃)); ¹³C NMR (125 MHz, CD₃OD) δ 175.7, 173.7, 103.5, 83.3, 77.9, 77.0, 72.5, 62.6, 57.0, 56.1, 52.4, 23.2, 19.4; HRMS m/z calcd for C₁₃H₂₃NO₈Na (M+Na⁺): 344.1315. Found 344.1313. Anal. Calcd for C₁₃H₂₃NO₈: C 48.59, H 7.21, N 4.36. Found: C 48.66, H 7.22, N 4.31.

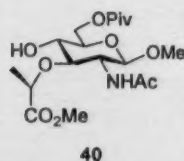


39

Methyl 2-acetamido-3-O-[(*R*)-1-carboxyethyl]-2-deoxy- β -D-glucopyranoside (39)

To a solution of compound **38** (0.94 g, 2.9 mmol) in a mixture of dioxane and methanol (60 mL, 1:1, v/v) was added dropwisely an aqueous 1M lithium hydroxide solution at room temperature until the mixture was adjusted to pH 10. The mixture was then stirred at room temperature for 8-10 h. The basicity of the mixture was kept between pH 9.5 to 10.5 by dropwise addition of 1M LiOH solution until the reaction was completed. The mixture was neutralized with amberlite IR-120 acidic resin. After filtration and concentration of the filtrate, the resulting pale oil residue was applied to a C18 reverse phase column and eluted with a gradient of 0 to 10% water in methanol to afford the desired compound **39** (0.89 g, 99%). $[\alpha]_D -8.8$ (c 1.3, CH₃OH); ¹H NMR (600 MHz, D₂O) δ 4.42 (d, 1H, $J_{1,2} = 8.5$ Hz, H-1), 4.31 (q, 1H, $J = 6.9$ Hz, CH(CO₂H,CH₃)), 3.92 (dd, 1H, $J_{6a,6b} = 12.4$ Hz, H-6a), 3.74 (dd, 1H, H-6b), 3.70 (dd, 1H, $J_{2,3} = 10$ Hz, H-2), 3.53 (dd, 1H, $J_{3,4} = 8.6$ Hz, H-3), 3.49 (dd, 1H, $J_{4,5} = 8.6$ Hz, H-4), 3.48 (s, 3H, OCH₃), 3.45 (ddd,

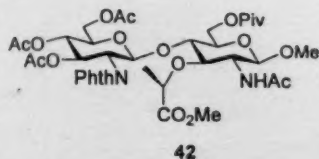
1H, $J_{5,6b} = 5.7$ Hz, $J_{5,6a} = 2.2$ Hz, H-5), 2.0 (s, 3H, NHCOCH_3), 1.37 (d, 3H, $J = 6.9$ Hz, $\text{CH}_3(\text{H}, \text{CO}_2\text{H})$); ^{13}C NMR (125 MHz, D_2O) δ 179.4, 175.4, 102.9, 83.0, 78.3, 76.5, 70.4, 61.6, 58.0, 55.4, 23.2, 19.5; ESI HRMS m/z calcd for $\text{C}_{12}\text{H}_{21}\text{NO}_8\text{Na}$ ($\text{M}+\text{Na}^+$): 330.1159. Found 330.1157.



Methyl 2-acetamido-2-deoxy-3-O-[(*R*)-1-(methoxycarbonyl)-ethyl]-6-O-pivaloyl- β -D-glucopyranoside (40)

To a solution of compound **38** (0.44 g, 1.36 mmol) in a mixture of dry dichloromethane (3 mL) and dry pyridine (4.5 mL) was added dropwise at 0 °C pivaloyl chloride (0.17 mL, 1.37 mmol). Then the mixture was stirred at 0 °C for 2-3 h. The mixture was diluted with dichloromethane, washed with sat. NaHCO_3 , water, brine and dried over anhydrous Na_2SO_4 . After filtration and concentration of the filtrate, the resulting residue was chromatographed using 2.5% methanol in dichloromethane to afford the desired compound **40** as a white solid (455 mg, 82%): $R_f = 0.55$ (20:1 CH_2Cl_2 - CH_3OH); $[\alpha]_D - 20.8$ (c 1.14, CHCl_3); ^1H NMR (600 MHz, CDCl_3) δ 6.5 (d, 1H, $J_{\text{NH},\text{H}-2} = 7.1$ Hz, NH), 4.64 (q, 1H, $J = 7.0$ Hz, $\text{CH}(\text{CO}_2\text{H}, \text{CH}_3)$), 4.56 (dd, 1H, $J_{6a,6b} = 12.1$ Hz, H-6a), 4.43 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1), 4.22 (dd, 1H, H-6b), 3.74 (s, 3H, COOCH_3), 3.62 (dd, 1H, $J_{3,4} = 8.2$ Hz, H-3), 3.57 (dd, 1H, $J_{2,3} = 10.5$ Hz, H-2), 3.48 (s, 3H, OCH_3), 3.39 (ddd, 1H, $J_{5,6a} = 4.0$ Hz, $J_{5,6b} = 2.4$ Hz, H-5), 3.35 (dd, 1H, $J_{4,5} = 9.6$ Hz, H-4), 2.03 (s, 3H, NHCOCH_3), 1.39 (d, 3H, $J = 7.0$ Hz, $\text{CH}_3(\text{H}, \text{CO}_2\text{H})$), 1.22 (s, 9H, $\text{C}(\text{CH}_3)_3$); ^{13}C NMR (125 MHz,

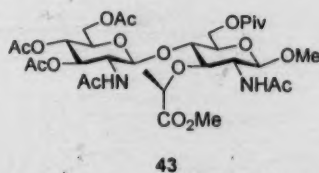
CDCl_3) δ 179.9, 175.4, 171.6, 102.6, 79.7, 74.4, 74.3, 71.4, 63.2, 56.6, 54.9, 52.1, 39.0, 27.2, 23.6, 19.1; HRMS m/z calcd for $\text{C}_{18}\text{H}_{31}\text{NO}_9\text{Na}$ ($\text{M}+\text{Na}^+$): 428.1891. Found: 428.1893; Anal. Calcd for $\text{C}_{18}\text{H}_{31}\text{NO}_9$: C 53.32, H 7.71, N 3.45. Found: C 53.00, H 7.55, N 3.44.



Methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido- β -glucopyranosyl-(1 $\rightarrow$ 4)-2-acetamido-2-deoxy-3-*O*-[(*R*)-1-(methoxycarbonyl)-ethyl]-6-*O*-pivaloyl- β -D-glucopyranoside (42)

To a solution of compound **40** (0.55 g, 1.36 mmol) and **41** (2.6 g, 5.4 mmol) in dry dichloromethane (14 mL) was added 4Å molecular sieves (3 g) and the mixture was stirred at room temperature for 10 minutes before cooled to -60°C . Then silver triflate (1.41 g, 5.4 mmol) was added at -60°C under argon and the mixture was stirred at this temperature 18 h before slow warming to room temperature over 2 h. The solution was washed with sat. NaHCO_3 , water, brine and dried over anhydrous Na_2SO_4 . Upon concentration of the filtrate, the residue was purified by silica gel chromatography using a gradient of 1 to 2% methanol in dichloromethane to yield the target compound **42** as a white solid (0.78 g, 70%); $R_f = 0.32$ (20:1 CH_2Cl_2 - CH_3OH); $[\alpha]_D -22.8$ (c 0.18, CHCl_3); ^1H NMR (600 MHz, CDCl_3) δ 7.86 (bs, 2H, ArH), 7.76 (m, 2H, ArH), 6.87 (d, 1H, $J_{\text{NH},\text{H}-2} = 7.1$ Hz, NH), 5.82 (dd, 1H, $J_{3',4'} = 9.0$ Hz, H-3'), 5.42 (d, 1H, $J_{1',2'} = 8.4$ Hz, H-1'), 5.18 (dd, 1H, $J_{4',5'} = 10.0$ Hz, H-4'), 4.68 (q, 1H, $J = 6.9$ Hz, $\text{CH}(\text{CO}_2\text{H}, \text{CH}_3)$), 4.46 (dd, 1H,

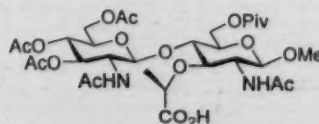
$J_{6a',6b'} = 12.4$ Hz, H-6a'), 4.36 (dd, 1H, $J_{6a,6b} = 12.2$ Hz, H-6a), 4.26 (dd, 1H, $J_{2',3'} = 10.5$ Hz, H-2'), 4.17 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1), 4.13 (dd, 1H, H-6b'), 3.89 (dd, 1H, $J_{4,5} = 8.8$ Hz, H-4), 3.86 (ddd, $J_{5',6a'} = 4.6$ Hz, $J_{5',6b'} = 2.2$ Hz, H-5'), 3.77 (s, 3H, COOCH₃), 3.67 (ddd, 1H, $J_{2,3} = 10.1$ Hz, H-2), 3.61 (dd, 1H, H-6b), 3.47 (dd, 1H, $J_{3,4} = 8.5$ Hz, H-3), 3.40 (s, 3H, OCH₃), 3.24 (ddd, 1H, $J_{5,6b} = 4.5$ Hz, $J_{5,6a} = 2.1$ Hz, H-5), 2.08 (s, 3H, COCH₃), 2.04 (s, 3H, COCH₃), 2.02 (s, 3H, COCH₃), 1.85 (s, 3H, COCH₃), 1.44 (d, 3H, $J = 6.9$ Hz, CH₃(H, CO₂H)), 1.20 (s, 9H, C(CH₃)₃); ¹³C NMR (125 MHz, CDCl₃) δ 177.3, 171.6, 170.4, 169.9, 169.5, 134.5, 134.4, 131.3, 131.2, 124.0, 123.7, 102.7, 97.3, 74.9, 72.8, 72.0, 70.5, 68.6, 61.9, 61.6, 56.2, 55.1, 54.5, 52.1, 38.8, 27.1, 23.5, 20.6, 20.6, 20.4, 18.6; ESI HRMS m/z calcd for C₃₈H₅₀N₂O₁₈Na (M+Na⁺): 845.2951. Found: 845.2957.



Methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- β -glucopyranosyl-(1 $\rightarrow$ 4)-2-acetamido-2-deoxy-3-*O*-[(*R*)-1-(methoxycarbonyl)-ethyl]-6-*O*-pivaloyl- β -D-glucopyranoside (43)

To a solution of compound **42** (250 mg, 0.3 mmol) in 95% ethanol (15.5 mL) was added hydrazine (115 μ L) and the mixture was stirred at 35 $^{\circ}$ C for 2 days. After that the reaction was quenched with several drops of acetic acid and evaporated to a residue. This residue was dissolved in a mixture of dry pyridine (3 mL) and acetic anhydride (1 mL) for and stirred at room temperature for several hours. The mixture was diluted with dichloromethane, washed with water, brine and dried over anhydrous Na₂SO₄. Filtration

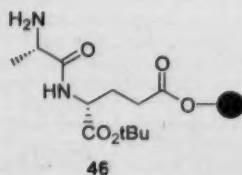
and concentration of the filtrate gave a residue, which was purified by silica gel chromatography (2.5% methanol in dichloromethane) to provide the target product **43** as a white solid (194 mg, 87%): $R_f = 0.28$ (20:1 CH_2Cl_2 - CH_3OH); $[\alpha]_D -36.4$ (c 0.11, CHCl_3); ^1H NMR (600 MHz, CD_3OD) δ 5.16 (dd, 1H, $J_{3',4'} = 9.2$ Hz, H-3'), 5.02 (dd, 1H, $J_{4',5'} = 9.8$ Hz, H-4'), 4.63 (q, 1H, $J = 7.0$ Hz, $\text{CH}(\text{CO}_2\text{H}, \text{CH}_3)$), 4.56 (d, 1H, $J_{1',2'} = 8.4$ Hz, H-1'), 4.50 (dd, 1H, $J_{6a,6b} = 11.9$ Hz, H-6a), 4.37 (dd, 1H, $J_{6a',6b'} = 12.5$ Hz, H-6a'), 4.24 (d, 1H, $J_{1,2} = 7.9$ Hz, H-1), 4.06 (dd, 1H, H-6b'), 4.02 (dd, 1H, H-6b), 3.92 (dd, 1H, $J_{2,3'} = 10.5$ Hz, H-2'), 3.75 (dd, 1H, $J_{4,5} = 8.7$ Hz, H-4), 3.72 (s, 3H, COOCH_3), 3.69 (ddd, 1H, $J_{5',6a'} = 4.3$ Hz, $J_{5',6b'} = 2.3$ Hz, H-5'), 3.66 (dd, 1H, $J_{2,3} = 10.1$ Hz, H-2), 3.54 (dd, 1H, $J_{3,4} = 8.4$ Hz, H-3), 3.48 (ddd, 1H, $J_{5,6b} = 6.0$ Hz, $J_{5,6a} = 2.3$ Hz, H-5), 3.38 (s, 3H, OCH_3), 2.02 (s, 3H, COCH_3), 2.00 (s, 3H, COCH_3), 1.98 (s, 6H, COCH_3), 1.90 (s, 3H, COCH_3), 1.37 (d, 3H, $J = 7.0$ Hz, $\text{CH}_3(\text{H}, \text{CO}_2\text{H})$), 1.20 (s, 9H, $\text{C}(\text{CH}_3)_3$); ^{13}C NMR (125 MHz, CD_3OD) δ 179.8, 176.3, 173.9, 173.5, 172.3, 171.9, 171.3, 103.6, 101.6, 79.5, 78.5, 76.7, 74.4, 73.8, 73.0, 70.0, 63.8, 63.8, 63.1, 40.1, 57.3, 56.0, 52.8, 52.8, 28.0, 23.7, 23.7, 23.3, 23.3, 21.3, 21.1, 19.4, 19.4; HRMS m/z calcd for $\text{C}_{32}\text{H}_{50}\text{N}_2\text{O}_{17}\text{Na}$ ($\text{M} + \text{Na}^+$): 757.3001. Found: 757.2996. Anal. Calcd for $\text{C}_{32}\text{H}_{50}\text{N}_2\text{O}_{17}$: C 52.31, H 6.86, N 3.81. Found: C 52.04, H 6.87, N 3.80.



44

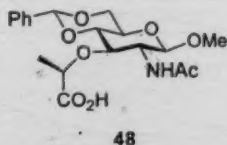
Methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- β -glucopyranosyl-(1 $\rightarrow$ 4)-2-acetamido-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-6-*O*-pivaloyl- β -D-glucopyranoside (44)

Anhydrous lithium iodide solid (240 mg, 1.8 mmol) was added to a solution of compound **43** (120 mg, 0.16 mmol) in dry pyridine (4 mL). The mixture was stirred at 100 °C for 2-3 days under argon and monitored by TLC. After the removal of pyridine, the residue was dissolved in a mixture of water/dichloromethane and acidified with 1M HCl solution to pH 2. Extraction with dichloromethane and concentration of the extract gave a residue, which was purified by silica gel chromatography using 5% methanol in dichloromethane containing 1% acetic acid to provide the desired compound **44** as a white solid (101 mg, 85%); $R_f = 0.36$ (100:10:1 CH₂Cl₂-CH₃OH-AcOH); $[\alpha]_D -45.5$ (c 0.67, CHCl₃); ¹NMR (600 MHz, CD₃OD) δ 5.28 (dd, 1H, $J_{3',4'} = 10.0$ Hz, H-3'), 5.00 (dd, 1H, $J_{4',5'} = 9.8$ Hz, H-4'), 4.74 (d, 1H, $J_{1',2'} = 8.4$ Hz, H-1'), 4.61 (dd, 1H, $J_{6a,6b} = 12.0$ Hz, H-6a), 4.56 (s, broad, 1H, CH (CO₂H,CH₃)), 4.39 (dd, 1H, $J_{6a',6b'} = 12.4$ Hz, H-6a'), 4.28 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1), 4.12 (d, broad, H-6b'), 4.04 (dd, 1H, $J_{5,6b} = 6.2$ Hz, H-6b), 3.81 (m, 3H, H-2', H-2, H-4), 3.76 (ddd, 1H, $J_{5',6a'} = 4.0$ Hz, $J_{5',6b'} = 2.2$ Hz, H-5'), 3.61 (dd, 1H, $J_{2,3} = 9.0$ Hz, H-3), 3.54 (m, 1H, H-5), 3.39 (s, 3H, OCH₃), 2.02 (s, 3H, COCH₃), 1.99 (s, 3H, COCH₃), 1.98 (s, 3H, COCH₃), 1.96 (s, 3H, COCH₃), 1.92 (s, 3H, COCH₃), 1.44 (d, 3H, $J = 6.8$ Hz, CH₃(H, CO₂H)), 1.23 (s, 9H, C(CH₃)₃); ¹³C NMR (125 MHz, CD₃OD) δ 179.4, 173.7, 172.3, 171.8, 171.3, 103.6, 101.5, 79.9, 78.6, 74.4, 73.7, 73.0, 70.0, 63.7, 62.9, 56.9, 56.2, 56.2, 49.2, 39.9, 27.6, 23.2, 22.9, 20.7, 20.6, 20.5, 19.3; ESI HRMS m/z calcd for C₃₁H₄₈N₂O₁₇Na: 743.2845. Found: 743.2848.



Dipeptide 46 (L-Ala-D-Glu-OtBu) on resin

Dipeptide (Fmoc-L-Ala-D-Glu-OtBu) on resin was prepared by using standard Fmoc chemistry, and Fmoc-D-Glu-OtBu **45** was loaded to Wang resin by asymmetric anhydride approach. The removal of Fmoc group by 20% piperidine in DMF provided the desired dipeptide **46** on resin for the subsequent coupling with muramic acid.

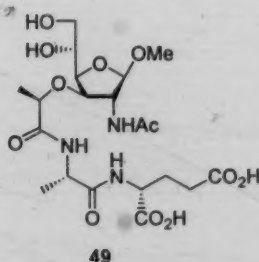


Methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-[(*R*)-1-carboxyethyl]-β-*D*-glucopyranoside (**48**)

Method A: Compound **39** (390 mg, 1.26 mmol) was dissolved in dry pyridine (6 mL) and benzyl dibromide (130 μ L, 1.4 mmol) was added. The mixture was stirred at 115 °C for 2 hours. After the removal of solvent by co-evaporation with toluene, the residue was washed by methanol to give a crude product, which was further purified by chromatography on silica gel using 100:10:1 dichloromethane/methanol/acetic acid as eluent to afford the pure compound **48** as a white solid (457 mg, 91%).

Method B: To a mixture of compound **38** (243 mg, 0.756 mmol) in dry acetonitrile were successively added benzylaldehyde dimethyl acetal (120 μ L, 0.8 mmol) and a catalytic amount of *p*-toluenesulfonic acid (2.6 mg). The mixture was then stirred at 50 °C for 2 h. After the removal of solvent, the residue was dissolved in ethyl acetate and extracted with water, brine. The extracts were concentrated and the residue was redissolved in dioxane and methanol. To this mixture, several drops of 1M Lithium were added to adjust the pH of solution to 10. The mixture was then stirred at room temperature for 8-10 h. The

basicity of the mixture was kept between pH 9.5 to 10.5 by dropwise addition of 1M LiOH solution until the reaction was completed. The mixture was neutralized with amberlite IR-120 acidic resin. After filtration and concentration of the filtrate, the resulting residue was purified by silica gel chromatography using dichloromethane in methanol as eluent to afford the desired compound **48** as a white solid (257 mg, 86% over two steps). $R_f = 0.12$ (100:10:1 CH_2Cl_2 - CH_3OH -AcOH); ^1H NMR (500 MHz, CD_3OD) δ 7.23 (m, 2H, Ar), 7.18 (m, 3H, ArH), 5.62 (s, 1H, PhCH), 4.45 (d, 1H, $J_{1,2} = 8.1$ Hz), 4.37 (q, 1H, $J = 7.0$ Hz, $\text{CH}(\text{CO}_2\text{H}, \text{CH}_3)$), 4.28 (dd, 1H, $J = 10.4$ Hz, 5.1 Hz), 3.58-3.84 (m, 5H), 3.44 (s, 3H, OCH_3), 1.99 (s, 3H, NHCOCH_3), 1.43 (d, 3H, $J = 7.0$ Hz, $\text{CH}_3(\text{H}, \text{CO}_2\text{H})$); ^{13}C NMR (125 MHz, CD_3OD) δ 175.0, 139.1, 129.8, 129.1, 127.2, 105.8, 102.6, 83.8, 79.6, 77.8, 69.7, 68.0, 57.2, 56.8, 23.3, 20.0; ESI HRMS m/z calcd for $\text{C}_{19}\text{H}_{25}\text{NO}_8\text{Na}$ ($\text{M} + \text{Na}^+$): 418.1472. Found: 418.1475.

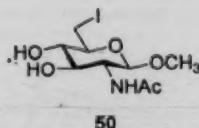


Methyl β -D-muramyl dipeptide (**49**)

To a solution of **46** on resin (0.1 mmol) in dry DMF, compound **48** (83 mg, 0.21 mmol), HBTU (75.9 mg, 0.20 mmol), HOBt (28.4 mg, 0.21 mmol) and DIPEA (73 μL , 0.42 mmol) were successively added at RT. The mixture was agitated by argon bubbles RT for 2 h. After the removal of solvent by vacuum filtration, the resin was washed by DMF and dichloromethane for three times and dried under vacuum filtration for 5 min. Next, the

resin was treated with a mixture of TFA/water/TIPS (95/2.5/2.5) for 18 h at RT. After filtration and concentration of the filtrate, the residue was washed by ether and applied to a C-18 reverse phase column on HPLC using water and acetonitrile containing 0.1% acetic acid and the fraction was lyophilized to afford compound **49** as the white foam (43 mg). $[\alpha]_D +6.3$ (c 0.69, CH_3OH); ^1H NMR (500 MHz, D_2O) δ 4.98 (s, 1H, H-1), 4.44 (t, 1H, $J = 5.0$ Hz, CH_2), 4.42 (q, 1H, $J = 7.1$ Hz, CHCH_3); 4.28 (q, 1H, $J = 7.0$ Hz, CHCH_3), 4.24 (dd, 2H, $J_{4,5} = 8.9$ Hz, $J = 4.9$ Hz, H-2, H-4 and $\text{CH}(\text{CO}_2\text{H})$), 4.16 (d, 1H, $J = 4.9$ Hz, H-3), 4.05 (ddd, 1H, $J_{5,6b} = 5.7$ Hz, $J_{5,6a} = 2.8$ Hz, H-5), 3.87 (dd, 1H, $J_{6a,6b} = 12.0$ Hz, H-6a), 3.72 (dd, 1H, H-6b), 3.42 (s, 3H, OCH_3), 2.48 (t, 2H, $J = 7.6$ Hz, CH_2), 2.24 (m, 1H, CH_2), 2.00 (s, 3H, NHCOCH_3), 1.99 (m, 1H, $-\text{CH}_2$), 1.44 (d, 3H, $J = 7.2$ Hz, CHCH_3), 1.39 (d, 3H, $J = 6.8$ Hz, CHCH_3); ^{13}C NMR (125 MHz, D_2O) δ 118.0, 176.3, 176.0, 175.6, 174.9, 108.3 (C-1), 82.5, 81.4, 76.9, 70.1, 64.5, 60.2, 56.3, 53.1, 50.3, 30.9, 26.8, 22.6, 18.0, 17.9; ESI HRMS m/z calcd for $\text{C}_{20}\text{H}_{32}\text{N}_3\text{O}_{12}(\text{M}-\text{H}^+)$: 506.1980. Found: 506.1981.

Chapter Four-Part I
Synthesis of Phytosphingosines via OCM

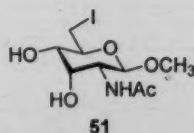


Methyl 2-acetamido-2,6-dideoxy-6-iodo- β -D-glucopyranoside (50)

Method A: To a solution of methyl 2-acetamido-2-deoxy- β -D-glucopyranoside **10** (1.175 g, 5 mmol) and PPh_3 (3.93 g, 15 mmol) in anhydrous pyridine (55 mL) was added portionwise carbon tetraiodide (3.9 g, 7.5 mmol) at 0 °C. The mixture was warmed to 40 °C and stirred for 5-6 h (monitored by TLC). The reaction was quenched by addition of methanol (10 mL), and the mixture was concentrated and filtered. The filtrate was concentrated and separated by chromatography on silica gel using 3% methanol in dichloromethane as eluent to give a pale yellow solid residue. This residue was dissolved in methanol (20 mL) and Dowex MR-3 mixed-bed ion-exchange resin (10 g) was added. The mixture was stirred at RT for 20 min and filtered off. Subsequent concentration of the filtrate gave the desired compound **50** as a white and pure solid (1.24 g, 74%)

Method B: Mesyl chloride (1.6 mL, 20.6 mmol) was added dropwise to a solution of methyl 2-acetamido-2-deoxy- β -D-glucopyranoside **10** (4.7 g, 20 mmol) in dry pyridine (150 mL) at -10 °C. The mixture was stirred at -10 °C for 18 h. After concentration, the residue was redissolved in dry acetone (160 mL) and NaI (9 g, 60 mmol) was added. The mixture was stirred at reflux for 18 h. Filtration followed by the removal of solvent gave a residue, which was separated by silica gel chromatography using 3% methanol in dichloromethane as eluent to afford a solid crude. This crude product was dissolved in

methanol (30 mL) and Dowex MR-3 mixed bed ion-exchange resin (10 g) was added. The mixture was stirred at RT for 20 min and filtered off. Subsequent concentration of the filtrate afforded the desired compound **50** as a white and pure solid (5.4 g, 78%): $R_f = 0.59$ (5:1 $\text{CH}_2\text{Cl}_2\text{-CH}_3\text{OH}$); $[\alpha]_D -2.2$ (c 0.68, CH_3OH); $^1\text{H NMR}$ (500 MHz, CD_3OD) δ 4.34 (d, 1H, $J_{1,2} = 8.5$ Hz, H-1), 3.63 (d, 1H, $J_{2,3} = 10.3$ Hz, H-2), 3.61 (dd, 1H, $J_{6a,6b} = 10.7$ Hz, H-6a), 3.46 (s, 3H, OCH_3), 3.44 (dd, 1H, $J_{3,4} = 8.6$ Hz, H-3), 3.32 (dd, 1H, H-6b), 3.18 (dd, 1H, $J_{4,5} = 9.2$ Hz, H-4), 3.10 (ddd, 1H, $J_{5,6b} = 7.4$ Hz, $J_{5,6a} = 2.4$ Hz, H-5), 1.98 (s, 3H, NHCOCH_3); $^{13}\text{C NMR}$ (125 MHz, CD_3OD) δ 173.8, 103.3, 76.6, 75.9, 75.7, 57.4, 57.0, 22.9, 6.4; ESI HRMS m/z calcd for $\text{C}_9\text{H}_{16}\text{NO}_5\text{INa}$ ($\text{M}+\text{Na}^+$): 367.9966. Found 367.9970. Anal. Calcd for $\text{C}_9\text{H}_{16}\text{NO}_5\text{I}$: C 31.32, H 4.67, N 4.06. Found: C 32.61, H 4.93, N 4.15.

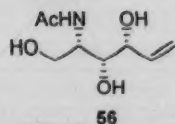


Methyl 2-acetamido-2,6-dideoxy-6-iodo- β -D-allopyranoside (**51**)

Method A: To a solution of methyl 2-acetamido-2-deoxy- β -D-allopyranoside **14** (235 mg, 1 mmol) in anhydrous pyridine (10 mL) at 0°C , PPh_3 (786 mg, 3 mmol) and carbon tetraiodide (780 mg, 2.5 mmol) were successively added in portions. The mixture was warmed to 40°C and stirred for 5-6 h (the reaction was monitored by TLC). Then the reaction was quenched by addition of methanol (2 mL). After concentration, filtration and solvent removal, the resulting residue was separated by chromatography on silica gel using 3% methanol in dichloromethane as eluent to give a pale yellow solid residue. This residue was dissolved in methanol (5 mL) and Dowex MR-3 mixed-bed ion-exchange

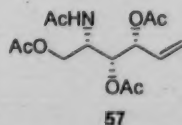
resin (2 g) was added. The mixture was stirred at RT for 20 min and filtered off. Subsequent concentration of the filtrate gave the desired compound **51** as a white and pure solid (245 mg, 71%).

Method B: Mesyl chloride (1.23 mL, 16.2 mmol) was added dropwise to a solution of methyl 2-acetamido-2-deoxy- β -D-allopyranoside **14** (3.47 g, 14.8 mmol) in dry pyridine (100 mL) at $-30\text{ }^{\circ}\text{C}$. The mixture was stirred at $-30\text{ }^{\circ}\text{C}$ for 18 h. After concentration, the residue was dissolved in dry acetone (120 mL). To the above solution, NaI (6.75 g, 45 mmol) was added and the mixture was stirred at reflux for 18 h. After filtration and solvent removal, the residue was separated by silica gel chromatography using 3 % methanol in dichloromethane as eluent to give a solid residue. The residue was dissolved in methanol (20 mL) and Dowex MR-3 mixed bed ion-exchange resin (10 g) was added. The mixture was stirred at RT for 20 min and filtered off. Subsequent concentration of the filtrate afforded the desired compound **51** as a white solid (4.39 g, 86%): $R_f = 0.48$ (10:1 $\text{CH}_2\text{Cl}_2\text{-CH}_3\text{OH}$); $[\alpha]_D -47.2$ (c 1.12, CH_3OH); $^1\text{H NMR}$ (500 MHz, CD_3OD) δ 4.60 (d, 1H, $J_{1,2} = 8.6$ Hz, H-1), 3.93 (dd, 1H, $J_{3,4} = 2.9$ Hz, H-3), 3.77 (dd, 1H, $J_{2,3} = 2.8$ Hz, H-2), 3.59 (dd, 1H, $J_{6a,6b} = 10.5$ Hz, H-6a), 3.55 (ddd, 1H, $J_{5,6b} = 7.7$ Hz, $J_{5,6a} = 2.4$ Hz, H-5), 3.47 (s, 3H, OCH_3), 3.36 (dd, 1H, $J_{4,5} = 9.3$ Hz, H-4), 3.27 (dd, 1H, H-6b), 1.98 (s, 3H, NHCOCH_3); $^{13}\text{C NMR}$ (125 MHz, CD_3OD) δ 173.0, 101.2, 74.4, 72.6, 71.4, 56.9, 55.0, 22.6, 7.5; ESI HRMS m/z calcd for $\text{C}_9\text{H}_{17}\text{NO}_5\text{I}$ ($\text{M}+\text{H}^+$): 346.0146. Found 346.0146. Anal. Calcd for $\text{C}_9\text{H}_{16}\text{NO}_5\text{I}$: C 31.32, H 4.67, N 4.06. Found: C 31.85, H 5.01, N 4.00.



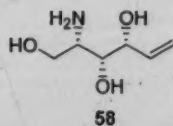
(2*S*, 3*R*, 4*R*)-2-acetamido-hex-5-ene-1,3,4-triol (56)

To a solution of compound **50** (2.40 g, 7 mmol) in a mixture of THF/water (30 mL, 4:1 v/v) was added freshly activated zinc powder (4.57 g, 70 mmol). The mixture was sonicated at 40-50 °C for 2 h and passed through a short silica pad, which was washed with acetone. After concentration, the residue was dissolved in dry methanol (20 mL) and NaBH₄ (1.32 g, 35 mmol) was added in portions at 0 °C. The reaction was continued from 0 °C to RT over 18 h and quenched by addition of acetic acid (1 mL). After filtration through a short silica pad and concentration, the residue was purified by chromatography on silica gel using a gradient of 10% to 15% methanol in dichloromethane, affording the desired product **56** as a white solid (1.2 g, 91%): *R_f* = 0.46 (5:1 CH₂Cl₂-CH₃OH); [α]_D -5.5 (*c* 0.62, CH₃OH); ¹H NMR (500 MHz, CD₃OD) δ 5.90 (ddd, 1H, *J*_{5,6t} = 17.1 Hz, *J*_{5,6c} = 10.5 Hz, H-5), 5.29 (ddd, 1H, *J*_{6t,6c} = 1.6 Hz, H-6t), 5.16 (ddd, 1H, H-6c), 4.02 (ddd, 1H, *J*_{2,3} = 2.7 Hz, H-2), 3.96 (ddd, 1H, *J*_{4,5} = 6.9 Hz, *J*_{4,6} = 1.2 Hz, H-4), 3.67 (dd, 1H, *J*_{3,4} = 7.1 Hz, H-3), 3.60 (dd, 1H, *J*_{1a,1b} = 10.8 Hz, *J*_{1a,2} = 6.9 Hz, H-1a), 3.52 (dd, 1H, *J*_{1b,2} = 6.0 Hz, H-1b), 1.96 (s, 3H, NHCOCH₃); ¹³C NMR (125 MHz, CD₃OD) δ 173.3, 138.9, 117.1, 74.8, 73.7, 62.8, 52.2, 22.9; ESI HRMS *m/z* calcd for C₈H₁₅NO₄Na (M+Na⁺): 212.0893. Found: 212.0895.



(2*S*, 3*R*, 4*R*)-2-Acetamido-1,3,4-tri-*O*-acetyl-hex-5-ene-1,3,4-triol (57)

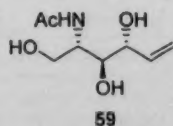
To a solution of compound **56** (190 mg, 1 mmol) in dry pyridine (2 mL), acetic anhydride (0.15 mL) was added dropwise. The mixture was stirred at RT for 18 h and diluted with dichloromethane, washed with water, brine and dried over anhydrous Na_2SO_4 . The removal of solvent provided the pure target product **57** as a colorless syrup (306 mg, 97%); $R_f = 0.18$ (1:4 hexane-ethyl acetate); $[\alpha]_D -19.2$ (c 2.4, CHCl_3); ^1H NMR (600 MHz, CDCl_3) δ 5.78 (ddd, 1H, $J_{5,6t} = 17.1$ Hz, $J_{5,6c} = 10.5$ Hz, H-5), 5.69 (d, 1H, $J_{\text{NH},2} = 9.5$ Hz), 5.39 (ddd, 1H, $J_{4,5} = 6.3$ Hz, $J_{4,6} = 1.1$ Hz, H-4), 5.37 (d, 1H, H-6t), 5.34 (d, 1H, H-6c), 5.19 (dd, 1H, $J_{3,4} = 7.3$ Hz, H-3), 4.55 (ddd, 1H, $J_{2,3} = 3.5$ Hz, H-2), 4.01 (dd, 1H, $J_{1a,1b} = 11.3$ Hz, $J_{1a,2} = 6.3$ Hz, H-1a), 3.97 (dd, 1H, $J_{1b,2} = 5.9$ Hz, H-1b), 2.10 (s, 3H, COCH_3), 2.07 (s, 3H, COCH_3), 2.05 (s, 3H, COCH_3), 2.02 (s, 3H, NHCOCH_3); ^{13}C NMR (125 MHz, CDCl_3) δ 170.5, 169.8, 169.7, 169.6, 131.3, 120.6, 73.0, 72.0, 62.9, 47.5, 23.3, 20.9, 20.7, 20.6; ESI HRMS m/z calcd for $\text{C}_{14}\text{H}_{21}\text{NO}_7\text{Na}$ ($\text{M}+\text{Na}^+$): 338.1210. Found: 338.1208.



(2*S*, 3*R*, 4*R*)-2-Amino-hex-5-ene-1,3,4-triol (58**)**

Triol **56** (100 mg, 0.53 mmol) was dissolved in 1M barium hydroxide solution (3 mL) and the mixture was heated at 90 °C for 18 h. After concentration, the residue was purified by silica gel chromatography using dichloromethane/methanol/ammonium hydroxide (100:15:2) as eluent to give the target compound **58** as a colorless syrup (69 mg, 88%); $R_f = 0.21$ (100:40:1 CH_2Cl_2 - CH_3OH); $[\alpha]_D +5.9$ (c 0.67, CH_3OH); ^1H (500 MHz, CD_3OD) δ 6.0 (ddd, 1H, $J_{5,6t} = 17.2$ Hz, $J_{5,6c} = 10.5$ Hz, H-5), 5.37 (dd, 1H, H-6t),

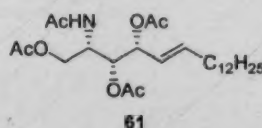
5.24 (dd, 1H, H-6c), 4.21 (dddd, $J_{4,5} = 5.8$ Hz, $J_{4,6t} = J_{4,6c} = 1.6$ Hz, H-4), 3.80 (dd, 1H, $J_{1a,1b} = 11.6$ Hz, $J_{1a,2} = 4.6$ Hz, H-1a), 3.72 (dd, 1H, $J_{1b,2} = 7.1$ Hz, H-1b), 3.70 (dd, 1H, $J_{3,4} = 2.8$ Hz, H-3), 3.38 (ddd, 1H, $J_{2,3} = 5.0$ Hz, H-2); ^{13}C NMR (125 MHz, CD_3OD) δ 138.9, 117.0, 74.9, 70.9, 60.7, 56.9; ESI HRMS m/z calcd for $\text{C}_6\text{H}_{14}\text{NO}_3$ ($\text{M}+\text{H}^+$): 148.0968. Found: 148.0969.



(2*S*, 3*S*, 4*R*)-2-Acetamido-hex-5-ene-1,3,4-triol (59)

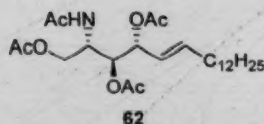
To a solution of allopyranoside **59** (345 mg, 1 mmol) in a mixture of THF/water (5 mL, 4:1 v/v) was added freshly activated zinc powder (653 mg, 10 mmol). The mixture was sonicated at 40–50 °C for 2 h and then passed through a short silica pad, which was washed with acetone. After concentration, the residue was dissolved in dry methanol (3 mL) and NaBH_4 (190 mg, 5 mmol) was added in portions at 0 °C. The mixture was stirred from 0 °C to RT over 18 h. Then the reaction was quenched by addition of acetic acid (0.5 mL). After filtration through a short silica pad and concentration, the residue was purified by silica gel chromatography using a gradient of 10% to 15% methanol in dichloromethane, affording the desired product **59** as a white solid (168 mg, 89%): $R_f = 0.17$ (10:1 CH_2Cl_2 - CH_3OH); $[\alpha]_D +8.4$ (c 0.63, CH_3OH); ^1H NMR (500 MHz, CD_3OD) δ 5.98 (ddd, 1H, $J_{5,6t} = 17.2$ Hz, $J_{5,6c} = 10.5$ Hz, H-5), 5.27 (ddd, 1H, $J_{6t,6c} = 1.7$ Hz, H-6t), 5.17 (ddd, 1H, H-6c), 4.08 (ddd, 1H, $J_{4,5} = 5.5$ Hz, $J_{4,6} = 1.4$ Hz, H-4), 4.02 (ddd, 1H, $J_{2,3} = 6.0$ Hz, H-2), 3.76 (dd, 1H, $J_{1a,1b} = 11.3$ Hz, $J_{1a,2} = 4.3$ Hz, H-1a), 3.71 (dd, 1H, $J_{1b,2} = 5.7$ Hz, H-1b), 3.64 (dd, 1H, $J_{3,4} = 5.2$ Hz, H-3), 1.96 (s, 3H, NHCOCH_3); ^{13}C NMR (125

MHz, CD₃OD) δ 173.2 138.7, 116.5, 75.4, 74.8, 62.0, 53.8, 49.5, 22.8; ESI HRMS m/z calcd for C₈H₁₅NO₄Na (M+Na⁺): 212.0893. Found 212.0891.



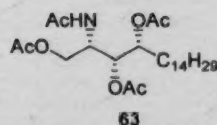
(2*S*,3*R*,4*R*)-2-Acetamido-1,3,4-tri-*O*-acetyl-(5*E*)-octadecene-1,3,4-triol (61)

To a solution of compound **57** (28 mg, 0.09mmol) and 1-tetradecene (0.112 mL, 0.445 mmol) in dry dichloromethane (2 mL), Grubbs' second generation catalyst (20%, 15.3 mg) was added in portionwise (protocol B) at reflux. The reaction was continued for 18 h under gentle reflux. After concentration, the residue was chromatographed on silica gel using hexane/ethyl acetate (1:1 to 1:2) as eluent to yield the desired compound **61** as a white solid (37 mg, 86%): R_f = 0.41 (1:5 hexane-ethyl acetate); $[\alpha]_D$ -26.6 (c 0.76, CHCl₃); ¹H NMR (500 Hz, CDCl₃) δ 5.82 (ddd, 1H, $J_{6,7a}$ = 6.7 Hz, $J_{6,7b}$ = 4.1 Hz, H-6), 5.69 (d, 1H, $J_{NH,H-2}$ = 9.5 Hz, NH), 5.32-5.38 (m, 2H, $J_{5,6}$ = 14.7 Hz, H-5 and H-4), 5.17 (dd, 1H, J = 7.4 Hz, J = 3.4 Hz, H-3), 4.52 (dddd, 1H, J = 7.8 Hz, J = 3.2 Hz, H-2), 3.94-4.02 (dd, 2H, $J_{1a,1b}$ = 11.3 Hz, J = 6.4 Hz, H-1a and H-1b), 2.09 (s, 3H, COCH₃), 2.05 (s, 3H, COCH₃), 2.03 (s, 3H, COCH₃), 2.02 (s, 3H, NHCOCH₃), 2.02 (m, 2H, alkane CH₂), 1.32-1.40 (d, 2H, J = 6.1 Hz, alkane CH₂), 1.20-1.32 (m, 18H, alkane CH₂), 0.88 (t, 3H, J = 6.7 Hz, alkyl CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 170.7, 169.8, 169.8, 169.5, 138.9, 122.6, 73.2, 72.3, 62.9, 47.5, 32.3, 31.9, 29.7, 29.6, 29.6, 29.6, 29.4, 29.3, 29.2, 28.6, 23.2, 22.7, 21.0, 20.7, 20.6, 14.1; ESI HRMS m/z calcd for C₂₆H₄₅NO₇Na: 506.3088. Found: 506.3089.



(2*S*,3*S*,4*R*)-2-Acetamido-1,3,4-tri-*O*-acetyl-(5*E*)-octadecene-1,3,4-triol (62)

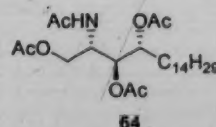
Triol **59** (28 mg, 0.148 mmol) was dissolved in dry pyridine (2 mL) and acetic anhydride (0.15 mL) was added dropwise. The mixture was stirred at RT for 18 h. Then the solution was diluted with dichloromethane and organic phase was washed with water, brine and dried over anhydrous Na₂SO₄. Removal of solvent gave a colorless syrup **60**, which was redissolved in dry dichloromethane (3 mL) followed by addition of 1-tetradecene (0.184 mL, 0.73 mmol). To the resulting mixture, Grubbs' second generation catalyst (20%, 25 mg) was added in four portions (protocol B) under reflux. The reaction was continued for 18 h at gentle reflux. The mixture was concentrated and purified by chromatography on silica gel using hexane/ethyl acetate (1:1 to 1:2) as eluent to yield the desired compound **62** as a white solid (52 mg, 73%): $R_f = 0.53$ (1:5 hexane-ethyl acetate); $[\alpha]_D +11.4$ (c 0.44, CHCl₃); ¹H NMR (500 Hz, CDCl₃) δ 5.83 (ddd, 2H, $J_{6,7} = 6.8$ Hz, 6.8 Hz, H-6, NH), 5.45 (dd, 1H, $J_{5,6} = 15.7$ Hz, H-5), 5.34 (dd, 1H, $J_{4,5} = 8.0$ Hz, H-4), 5.14 (dd, 1H, $J_{3,4} = 3.4$ Hz, H-3), 4.45 (dddd, 1H, $J_{NH, H-2} = 10.8$ Hz, $J_{2,3} = 8.0$ Hz, H-2), 4.30 (dd, 1H, $J_{1a,1b} = 11.6$ Hz, $J_{1a,2} = 4.6$ Hz, H-1a), 3.99 (dd, 1H, $J_{1b,2} = 3.3$ Hz, H-1b), 2.08 (s, 3H, COCH₃), 2.05 (s, 3H, COCH₃), 2.05 (s, 3H, COCH₃), 2.03 (s, 3H, NHCOCH₃), 2.02 (m, 2H, alkane CH₂), 1.34-1.42 (d, 2H, $J = 6.1$ Hz, alkane CH₂), 1.20-1.34 (m, 18H, alkane CH₂), 0.88 (t, 3H, $J = 6.7$ Hz, alkyl CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 170.7, 170.3, 170.0, 169.4, 138.9, 121.8, 74.0, 72.1, 62.8, 47.8, 32.4, 31.9, 29.7, 29.6, 29.6, 29.6, 29.4, 29.3, 29.2, 28.8, 23.4, 22.7, 21.1, 20.8, 20.7, 14.1; ESI HRMS m/z calcd for C₂₆H₄₅NO₇Na ($M+Na^+$): 506.3088. Found: 506.3088.



(2*S*,3*R*,4*R*)-2-Acetamido-1,3,4-tri-*O*-acetyl-octadecane-1,3,4-triol

(*D*-xylo-phytosphingosine tetraacetate) (63)

To a solution of compound **61** (20 mg, 0.04 mmol) in ethyl acetate (2 mL) was added a catalytic amount of palladium hydroxide on carbon (15 mg). The mixture was stirred at RT under an atmosphere of hydrogen for 18 h. After filtration, the filtrate was concentrated and dried under vacuum to provide the desired product **63** as a white solid in quantitative yield: $R_f = 0.45$ (1:4 hexane-ethyl acetate); $[\alpha]_D +6.0$ (c 0.62, CHCl_3); {lit.^[137b] $[\alpha]_D +6.3$ (c 0.86, CHCl_3)}; ^1H NMR (500 MHz, CDCl_3) δ 5.71 (d, 1H, $J_{\text{NH,H-2}} = 9.5$ Hz, NH), 5.16 (dd, 1H, $J_{3,4} = 6.5$ Hz, H-3), 5.06 (ddd, 1H, $J_{4,5} = 6.6$ Hz, H-4), 4.53 (dddd, $J_{2,3} = 4.4$ Hz, H-2), 4.05 (dd, 1H, $J_{1a,1b} = 11.4$ Hz, $J_{1a,2} = 6.0$ Hz, H-1a), 4.01 (dd, 1H, $J_{1b,2} = 5.7$ Hz, H-1b), 2.09 (s, 3H, COCH_3), 2.06 (s, 6H, COCH_3), 2.02 (s, 3H, NHCOCH_3), 1.55-1.64 (m, 2H, alkane CH_2), 1.20-1.35 (m, 24H, alkane CH_2), 0.88 (t, 3H, $J = 6.7$ Hz, alkyl CH_3); ^{13}C NMR (125 MHz, CDCl_3) δ 170.5, 170.5, 170.1, 169.8, 72.3, 71.9, 62.9, 48.0, 31.9, 30.5, 29.7, 29.7, 29.6, 29.6, 29.5, 29.4, 29.3, 29.2, 24.8, 23.2, 22.7, 20.9, 20.7, 20.6, 14.1; ESI HRMS m/z calcd for $\text{C}_{26}\text{H}_{47}\text{NO}_7\text{Na}$ ($\text{M}+\text{Na}^+$): 508.3245. Found: 508.3246.



(2*S*,3*S*,4*R*)-2-Acetamido-1,3,4-tri-*O*-acetyl-octadecane-1,3,4-triol (64)

(D-ribo-phytosphingosine tetraacetate)

To a solution of compound **62** (15 mg, 0.03 mmol) in ethyl acetate (2 mL) containing 5% acetic acid was added a catalytic amount of palladium hydroxide on carbon (12 mg). The mixture was stirred under an atmosphere of hydrogen at RT for several hours. After filtration, the filtrate was concentrated and dried under vacuum to afford the desired product **64** as a white solid (13.8 mg, 92%); $R_f = 0.58$ (1:5 hexane-ethyl acetate); $[\alpha]_D +25.8$ (c 1.4, CHCl_3); { lit.^[142a] $[\alpha]_D +27.8$ (c 0.8, CHCl_3), lit.^[142h] $[\alpha]_D +26.5$ (c 0.84, CHCl_3), lit.^[142j] $[\alpha]_D +26.2$ (c 2.0, CHCl_3)}; ^1H NMR (500 MHz, CDCl_3) δ 5.91 (d, 1H, $J_{\text{NH,H-2}} = 9.4$ Hz, NH), 5.10 (dd, 1H, $J_{3,4} = 3.1$ Hz, H-3), 4.94 (dt, 1H, $J_{4,5a} = 3.2$ Hz, $J_{4,5b} = 9.9$ Hz, H-4), 4.48 (dddd, 1H, $J_{2,3} = 8.2$ Hz, H-2), 4.29 (dd, 1H, $J_{1a,1b} = 11.7$ Hz, $J_{1a,2} = 5.0$ Hz, H-1a), 4.01 (dd, 1H, $J_{1b,2} = 3.1$ Hz, H-1b), 2.08 (s, 3H, COCH_3), 2.05 (s, 6H, COCH_3), 2.03 (s, 3H, NHCOCH_3), 1.55-1.64 (m, 2H, alkyl CH_2), 1.20-1.35 (m, 24H, alkyl CH_2), 0.88 (t, 3H, $J = 6.7$ Hz, alkyl CH_3); ^{13}C NMR (125 MHz, CDCl_3) δ 171.1, 170.8, 170.0, 169.7, 73.0, 72.1, 62.8, 47.7, 31.9, 29.7, 29.6, 29.6, 29.6, 29.5, 29.3, 29.3, 28.2, 25.5, 23.3, 22.7, 21.0, 20.7, 20.7, 14.1; ESI HRMS m/z calcd for $\text{C}_{26}\text{H}_{47}\text{NO}_7\text{Na}$: 508.3245. Found: 508.3247.

*Chapter Four - Part II***Synthesis of Sphingolipids via Asymmetric Aldol Condensation****General procedure B: aldol condensation**

To a solution of iminoglycinate **65**^[157] (6.0 mmol) in anhydrous dichloromethane (4 mL) at 0 °C was successively added a solution of ClTi(OEt)₃ (15.0 mmol) in anhydrous dichloromethane (8 mL), Et₃N (3.5 mL) and a solution of the aldehyde (6.6 mmol) in anhydrous dichloromethane (10 mL) in dropwise. The reaction mixture was stirred at 0 °C for 5–6 h, and quenched by addition of brine (30 mL). The mixture was diluted with dichloromethane (20 mL) and the organic phase was separated; the aqueous phase was extracted with more dichloromethane (2 × 50 mL), and organic layers were combined, dried and concentrated. Chromatography of the residue on silica gel (pretreated with 15% Et₃N in eluent) afforded the desired compounds.

For the condensation with acrolein, we also employed ClTi(OPr-*i*)₃ as reagent in a similar fashion (not shown in the experimental section). Ethyl and isopropyl esters were obtained as a 1:1 mixture of single diastereoisomers in 70% yield.

General procedure C: acid hydrolysis of imines

The imines (4.6 mmol) were dissolved in a mixture of 1.0 M HCl (32 mL) and THF (8 mL) and the mixture was stirred for 3 days at RT. The mixture was extracted with Et₂O (2 × 20 mL), and the organic phase containing mainly the auxiliary was evaporated. The aqueous phase was neutralized to pH 8 with a solution of sat. NaHCO₃, and extracted with dichloromethane until no more of the desired compound could be detected by TLC.

The combined extracts were dried and concentrated. Column chromatography of the residue on silica gel gave the desired amino esters.

General procedure D: reduction of the amino esters

Reduction with LiAlH_4

To a solution of amino ester (1.5 mmol) in anhydrous THF (35 mL) at 0 °C was added LiAlH_4 (15 mmol) in small portions under argon; the mixture was heated at reflux for 3 days. The reaction was cooled and the mixture was filtered through a thin pad of silica gel using CH_2Cl_2 -MeOH- NH_4OH (100:15:2) as eluent. After concentration, the residue was purified by column chromatography to provide the desired sphingoids.

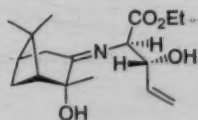
Reduction with LiBH_4

To a solution of amino ester (2.5 mmol) in methanol (15 mL) was added dropwise a solution of 2 M LiBH_4 in THF (7.5 mL, 15 mmol) at RT. The reaction was continued for 3 days and concentrated. The residue was loaded onto a thin pad of silica gel and eluted with CH_2Cl_2 -MeOH- NH_4OH (100:15:2) to afford the crude product. After concentration, the residue was purified by column chromatography to give the desired sphingoids.

General procedure E: oxidation with Dess-Martin periodinane (DMP)

To a suspension of DMP (8.0 mmol) in anhydrous dichloromethane (45 mL) was added pyridine (1.5 mL); the resulting slurry was stirred until all the reagents dissolved. A solution of alcohol (7.9 mmol) in anhydrous dichloromethane (20 mL) was added dropwise and the reaction was continued at room temperature for 5 hours. A solution of sat. brine (20 mL) and sat. Na_2SO_3 (20 mL) was added and the organic phase was

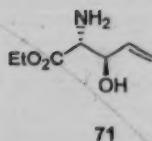
separated; the aqueous phase was extracted with more dichloromethane (3×50 mL), the organic extracts were combined, washed with water (1×100 mL), brine (1×40 mL), dried and evaporated. The desired aldehydes were obtained after column chromatography.



70

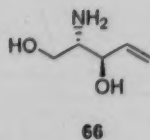
Ethyl {1R-[1 α ,2 β ,3(2R,3R),5 α]}-3-hydroxy-2-[(2-hydroxy-2,6,6-trimethylbicyclo[3,1,1]hept-3-ylidene)amino]-4-pentenoate (70)

The title compound was prepared from acrolein according to the general procedure B using ClTi(OEt)₃ as reagent. Compound **70** was obtained as a single diastereoisomer by chromatography on silica gel using hexane/EtOAc (4:1) as eluent and was found to contain ~10% isopropyl ester (72% yield): R_f = 0.43 (1:1 hexane–EtOAc); ¹H NMR (600 MHz, CDCl₃): δ 5.88 (ddd, 1H, J = 17.0 Hz, 10.5 Hz, 6.2 Hz), 5.38 (ddd, 1H, J = 17.0 Hz, 1.5 Hz, 1.5 Hz), 5.22 (ddd, 1H, J = 10.6 Hz, 1.5 Hz, 1.5 Hz), 4.62 (dd, 1H, J = 6.3 Hz, 6.3 Hz), 4.15–4.25 (m, 3H), 3.22 (broad s, 1H), 2.56 (dd, 1H, J = 18.0 Hz, 3.0 Hz), 2.53 (ddd, 1H, J = 18.0 Hz, 2.6 Hz, 2.6 Hz), 2.47 (broad s, 1H), 2.34 (ddd, 1H, J = 10.7 Hz, 6 Hz, 6 Hz, 2.3 Hz), 2.08 (dd, 1H, J = 5.9 Hz, 5.9 Hz), 2.03 (ddd, 1H, J = 6.0 Hz, 6.0 Hz, 3.0 Hz), 1.54 (d, 1H, J = 10.7 Hz), 1.50 (s, 3H), 1.33 (s, 3H), 1.24 (t, 3H, J = 7.1 Hz), 0.87 (s, 3H); for the isopropyl ester, all signals overlap with the ethyl ester except the signal at 5.05 (sept, 1H, J = 6.5 Hz). ¹³C NMR (CDCl₃, 100 MHz): δ 180.7, 169.9, 136.5, 117.0, 76.7, 73.8, 67.0, 61.1, 50.2, 38.6, 38.4, 34.1, 28.2, 27.9, 27.2, 22.7, 14.1. HRMS m/z Calcd for C₁₇H₂₈NO₄ (M + H⁺): 310.2018. Found 310.2018.



Ethyl (2*R*,3*R*)-2-amino-3-hydroxy-4-pentenoate (71)

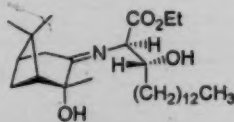
The title compound was prepared from **70** according to the general procedure **C**. Compound **71** (containing trace amounts of isopropyl ester) was obtained as colorless syrup by chromatography on silica gel using CH₂Cl₂/CH₃OH/NH₄OH (100:2.5:1) as eluent (82% yield): *R_f* = 0.27 (100:5:1 CH₂Cl₂-MeOH-NH₄OH); ¹H NMR (CD₃OD, 500 MHz): δ 5.73 (ddd, 1H, *J* = 16.7 Hz, 10.5 Hz, 6.5 Hz), 5.33 (ddd, 1H, *J* = 17.0 Hz, 1.6 Hz, 1.6 Hz), 5.21 (ddd, 1H, *J* = 10.5 Hz, 1.5 Hz, 1.5 Hz), 4.39 (dddd, 1H, *J* = 5.2 Hz, 5.2 Hz, 1.4 Hz, 1.4 Hz), 4.19 (dq, 1H, *J* = 10.8 Hz, 7.2 Hz), 4.16 (dq, 1H, *J* = 10.8 Hz, 7.2 Hz), 3.61 (d, 1H, *J* = 5.0 Hz), 1.26 (t, 3H, *J* = 7.0 Hz); ¹³C NMR (CD₃OD, 125 MHz): δ 173.2, 135.6, 117.3, 72.6, 61.2, 58.4, 14.2; HRMS *m/z* Calcd for C₇H₁₄NO₃ (M + H⁺): 160.0974. Found 160.0974. Anal. Calcd for C₇H₁₃NO₃: C 52.82, H 8.23, N 8.80. Found: C 52.92, H 8.36, N 8.31.



(2*S*,3*R*)-2-Aminopent-4-ene-1,3-diol (66)

The title compound was prepared from **71** according to the general procedure **D** using LiBH₄ as reducing reagent. Compound **66** was obtained as a single diastereoisomer by chromatography on silica gel using an eluent of CH₂Cl₂/CH₃OH/NH₄OH (100:10:1) as colorless syrup (74% yield): *R_f* = 0.27 (100:20:1 CH₂Cl₂-MeOH-NH₄OH); [α]_D +18.5 (*c*

0.75, MeOH); ^1H NMR (CD_3OD , 500 MHz): δ 5.90 (ddd, 1H, $J_{4,5t} = 17.0$ Hz, $J_{4,5c} = 10.5$ Hz, H-4), 5.30 (ddd, 1H, $J_{5t,5c} = 1.6$ Hz, H-5t), 5.21 (ddd, 1H, H-5c), 4.03 (dddd, 1H, $J_{3,4} = 6.2$ Hz, $J_{3,5t} = J_{3,5c} = 1.3$ Hz, H-3), 3.60 (dd, 1H, $J_{1a,1b} = 10.9$ Hz, $J_{1a,2} = 4.5$ Hz, H-1a), 3.50 (dd, 1H, $J_{1b,2} = 6.9$ Hz, H-1b), 2.77 (ddd, 1H, $J_{2,3} = 6.4$ Hz, H-2); ^{13}C NMR (CD_3OD , 125 MHz): δ 139.3, 117.1, 75.3, 64.2, 57.8; HRMS m/z Calcd for $\text{C}_5\text{H}_{11}\text{NO}_2\text{Na}$ ($\text{M} + \text{Na}^+$): 140.0682. Found 140.0680. Anal. Calcd for $\text{C}_5\text{H}_{11}\text{NO}_2$: C 51.26, H 9.46, N 11.96. Found: C 51.28, H 9.57, N 11.36.

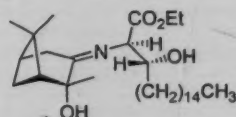


73a

Ethyl {1*R*-[1 α ,2 β ,3(2*R*,3*R*),5 α]}-3-hydroxy-2-[(2-hydroxy-2,6,6-trimethylbicyclo-[3,1,1]hept-3-ylidene)amino]hexadecanoate (73a)}

The title compound was prepared from **72a** according to the general procedure **B** using $\text{ClTi}(\text{OEt})_3$ as reagent. Compound **73a** (containing trace amounts of isopropyl ester) was obtained as a single diastereoisomer by chromatography on silica gel using hexane/EtOAc(6:1) as eluent (87% yield): $R_f = 0.29$ (4:1 hexane–EtOAc); ^1H NMR (CDCl_3 , 600 MHz): δ 4.19 (dq, 2H, $J = 7.2$ Hz, 2.8 Hz), 4.11 (d, 1H, $J = 6.3$ Hz), 4.07 (m, 1H), 3.01 (br s, 1H), 2.58 (dd, 1H, $J = 18.0$ Hz, 3.0 Hz), 2.53 (ddd, 1H, $J = 18.0$ Hz, 2.5 Hz, 2.5 Hz), 2.35 (dddd, 1H, $J = 10.7$ Hz, 6.0 Hz, 6.0 Hz, 2.3 Hz), 2.09 (dd, 1H, $J = 5.9$ Hz, 5.8 Hz), 2.04 (ddd, 1H, $J = 6.0$ Hz, 5.9 Hz, 3.0 Hz), 1.55 (d, 1H, $J = 10.7$ Hz), 1.50 (s, 3H), 1.33 (s, 3H), 1.25 (t, 3H, $J = 7.1$ Hz), 1.25–1.50 (m, 24H), 0.88 (t, 3H, $J = 7.0$ Hz), 0.87 (s, 3H); ^{13}C NMR (CDCl_3 , 125 MHz): δ 180.4, 170.6, 76.7, 72.6, 67.1,

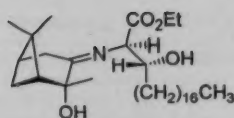
61.1, 50.1, 38.6, 38.4, 34.1, 32.8, 31.9, 29.7–29.6 (7C), 29.3, 28.3, 28.0, 27.3, 25.5, 22.8, 22.7, 14.2, 14.1. HRMS m/z Calcd for $C_{28}H_{52}NO_4$ ($M + H^+$): 466.3891; found 466.3893.



73b

Ethyl {1*R*-[1*α*,2*β*,3(2*R*,3*R*),5*α*]-3-hydroxy-2-[(2-hydroxy-2,6,6-trimethylbicyclo[3,1,1]hept-3-ylidene)amino]octadecanoate (73b)

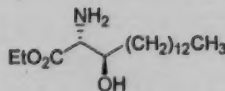
The title compound was prepared from **72b** according to general procedure **B** using $ClTi(OEt)_3$ as reagent. Compound **73b** (containing trace amounts of isopropyl ester) was obtained as a single diastereoisomer by chromatography on silica gel using hexane/EtOAc (6:1) as eluent (84% yield): R_f = 0.34 (4:1 hexane–EtOAc); ^1H NMR ($CDCl_3$, 600 MHz): δ 4.15 (dq, 2H, J = 7.2 Hz, 2.3 Hz), 4.09 (d, 1H, J = 6.0 Hz), 4.04 (m, 1H), 3.22 (br s, 1H), 2.53 (dd, 1H, J = 18.0 Hz, 2.5 Hz), 2.49 (ddd, 1H, J = 18.0 Hz, 2.5 Hz, 2.5 Hz), 2.32 (dddd, 1H, J = 10.8 Hz, 6.0 Hz, 5.9 Hz, 2.3 Hz), 2.06 (dd, 1H, J = 5.9 Hz, 5.9 Hz), 2.01 (ddd, 1H, J = 6.0 Hz, 5.9 Hz, 3.0 Hz), 1.52 (d, 1H, J = 10.7 Hz), 1.48 (s, 3H), 1.30 (s, 3H), 1.24 (t, 3H, J = 7.2 Hz), 1.25–1.50 (m, 28H), 0.85 (t, 3H, J = 6.8 Hz), 0.87 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 180.4, 170.5, 76.7, 72.6, 67.1, 61.1, 50.1, 38.6, 38.4, 34.1, 32.8, 31.9, 29.7–29.6 (9C), 29.3, 28.3, 28.0, 27.3, 25.5, 22.8, 22.7, 14.1, 14.1. HRMS m/z Calcd for $C_{30}H_{56}NO_4$ ($M + H^+$): 494.4204. Found 494.4202.



73c

Ethyl {1*R*-[1 α ,2 β ,3(2*R*,3*R*),5 α]}-3-hydroxy-2-[(2-hydroxy-2,6,6-trimethylbicyclo-[3,1,1]hept-3-ylidene)amino]icosanoate (73c)

The title compound was prepared from 72c according to the general procedure B using $\text{CITi}(\text{OEt})_3$ as reagent. Compound 73c (containing trace amounts of isopropyl ester) was obtained as a single diastereoisomer by chromatography on silica gel using hexane/EtOAc (6:1) as eluent (83% yield): $R_f = 0.34$ (4:1 hexane–EtOAc); ^1H NMR (CDCl_3 , 500 MHz): δ 4.17 (dq, 2H, $J = 7.1$ Hz, 2.0 Hz), 4.10 (d, 1H, $J = 6.1$ Hz), 4.07 (m, 1H), 3.20 (br s, 1H), 2.57 (dd, 1H, $J = 18.0$ Hz, 2.8 Hz), 2.50 (ddd, 1H, $J = 18.0$ Hz, 2.5 Hz, 2.5 Hz), 2.33 (dddd, 1H, $J = 10.8$ Hz, 6.0 Hz, 5.9 Hz, 2.3 Hz), 2.06 (dd, 1H, $J = 5.9$ Hz, 5.9 Hz), 2.02 (ddd, 1H, $J = 5.9$ Hz, 5.8 Hz, 2.8 Hz), 1.52 (d, 1H, $J = 10.9$ Hz), 1.50 (s, 3H), 1.31 (s, 3H), 1.22 (t, 3H, $J = 7.2$ Hz), 1.25–1.50 (m, 32H), 0.88 (t, 3H, $J = 7.0$ Hz), 0.87 (s, 3H); ^{13}C NMR (CDCl_3 , 125 MHz): δ 180.3, 170.5, 76.7, 72.6, 67.0, 61.1, 50.1, 42.9, 38.6, 38.4, 34.1, 32.7, 31.9, 29.3–29.7 (11C), 28.3, 28.0, 27.3, 25.5, 22.8, 22.6, 14.1, 14.1. HRMS m/z Calcd for $\text{C}_{32}\text{H}_{60}\text{NO}_4$ ($\text{M} + \text{H}^+$): 522.4517. Found 522.4519.

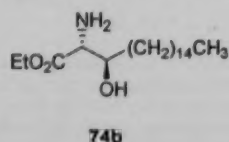


74a

Ethyl (2*R*,3*R*)-2-amino-3-hydroxyhexadecanoate (74a)

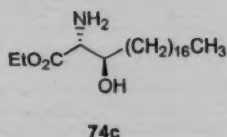
The title compound was prepared from 73a according to the general procedure C. Compound 74a was obtained by chromatography on silica gel using $\text{Et}_2\text{O}/\text{CH}_3\text{OH}$ (25:1)

as eluent (80% yield): $R_f = 0.31$ (20:1 Et₂O–MeOH); $[\alpha]_D -7.4$ (c 0.50, MeOH); ¹H NMR (CD₃OD, 600 MHz): δ 4.33 (dq, 1H, $J = 10.8$ Hz, 7.1 Hz), 4.29 (dq, 1H, $J = 10.8$ Hz, 7.1 Hz), 4.04 (d, 1H, $J = 3.3$ Hz), 3.99 (ddd, 1H, $J = 9.0$ Hz, 3.7 Hz, 3.7 Hz), 1.59 (m, 1H), 1.50 (m, 1H), 1.32 (t, 3H, $J = 7.1$ Hz), 1.25–1.40 (m, 22H), 0.89 (t, 3H, $J = 7.0$ Hz); ¹³C NMR (CD₃OD, 125 MHz): δ 168.3, 70.7, 63.5, 59.0, 33.6, 33.1, 30.8, 30.8, 30.8, 30.7, 30.7, 30.6, 30.4, 30.4, 26.9, 23.7, 14.4, 14.4. HRMS m/z Calcd for C₁₈H₃₈NO₃ ($M + H^+$): 316.2846. Found 316.2845. Anal. Calcd for C₁₈H₃₇NO₃: C 61.43, H 10.88, N 3.98. Found: C 61.47, H 11.07, N 3.73.



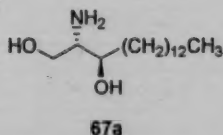
Ethyl (2*R*,3*R*)-2-amino-3-hydroxyoctadecanoate (74b)

The title compound was prepared from **73b** according to the general procedure C. Compound **74b** was obtained by chromatography on silica gel using Et₂O/CH₃OH (25:1) as eluent (84% yield): $R_f = 0.34$ (20:1 Et₂O–MeOH); $[\alpha]_D -9.6$ (c 0.57, MeOH); ¹H NMR (CD₃OD, 600 MHz): δ 4.32 (dq, 1H, $J = 10.8$ Hz, 7.2 Hz), 4.28 (dq, 1H, $J = 10.8$ Hz, 7.2 Hz), 4.04 (d, 2H, $J = 3.3$ Hz), 3.99 (ddd, 1H, $J = 8.7$ Hz, 5.0 Hz, 3.3 Hz), 1.59 (m, 1H), 1.50 (m, 1H), 1.32 (t, 3H, $J = 7.2$ Hz), 1.25–1.40 (m, 26H), 0.89 (t, 3H, $J = 7.0$ Hz); ¹³C NMR (CD₃OD, 125 MHz): δ 168.3, 70.7, 63.5, 58.9, 33.6, 33.1, 30.8–30.4 (10C), 26.9, 23.7, 14.4 (2C). HRMS m/z Calcd for C₂₀H₄₂NO₃: 344.3159 ($M + H^+$). Found 344.3159. Anal. Calcd for C₂₀H₄₁NO₃: C 63.21, H 11.14, N 3.69. Found: C 63.31, H 11.25, N 3.48.



Ethyl (2*R*,3*R*)-2-amino-3-hydroxyicosanoate (74c)

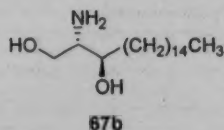
The title compound was prepared from **73c** according to the general procedure **C**. Compound **74c** was obtained by chromatography on silica gel using Et₂O/CH₃OH (25:1) as eluent (85% yield): $R_f = 0.35$ (20:1 Et₂O–MeOH); $[\alpha]_D -6.8$ (c 0.6, MeOH); ¹H NMR (CD₃OD, 600 MHz): δ 4.27 (dq, 1H, $J = 10.8$ Hz, 7.2 Hz), 4.23 (dq, 1H, $J = 10.8$ Hz, 7.2 Hz), 3.87 (ddd, 1H, $J = 9.0$ Hz, 8.9 Hz, 3.8 Hz), 3.76 (d, 1H, $J = 3.8$ Hz), 1.52–1.60 (m, 2H), 1.30 (t, 3H, $J = 7.2$ Hz), 1.25–1.40 (m, 30H), 0.89 (t, 3H, $J = 7.0$ Hz); ¹³C NMR (CD₃OD, 125 MHz): δ 174.4, 74.4, 62.0, 60.4, 33.8, 33.1, 30.9–30.5 (12C), 27.0, 23.7, 14.5, 14.4. HRMS m/z Calcd for C₂₂H₄₆NO₃ ($M + H^+$): 372.3472. Found 372.3471. Anal. Calcd for C₂₂H₄₅NO₃: C 64.75, H 11.36, N 3.43. Found: C 64.78, H 11.43, N 3.27.



(2*S*,3*R*)-2-Aminohexadecane-1,3-diol (67a)

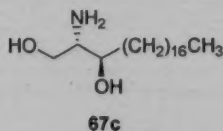
The title compound was prepared from **74a** according to the general procedure **D** using LiAlH₄ as reducing reagent. Compound **67a** was obtained a white solid by chromatography on silica gel using CH₂Cl₂/CH₃OH/NH₄OH (100:10:1) as eluent in 85% yield: $R_f = 0.21$ (100:10:2 CH₂Cl₂–MeOH–NH₄OH); $[\alpha]_D +8.7$ (c 1.0, MeOH); ¹H NMR (CD₃OD, 600 MHz): δ 3.72 (dd, 1H, $J_{1a,1b} = 10.9$ Hz, $J_{1a,2} = 4.2$ Hz, H-1a), 3.50 (ddd, 1H, $J_{3,4a} = 8.0$ Hz, $J_{3,4b} = 3.0$ Hz, H-3), 3.46 (dd, 1H, $J_{1b,2} = 7.6$ Hz, H-1b), 2.71 (ddd, 1H, $J_{2,3}$

= 5.4 Hz, H-2), 1.52 (m, 2H), 1.25–1.45 (m, 22H), 0.9 (t, 3H, $J = 7.0$ Hz); ^{13}C NMR (CD_3OD , 125 MHz): δ 74.0, 64.3, 58.1, 34.4, 33.1, 30.8–30.7 (8C), 27.0, 23.7, 14.4. HRMS m/z Calcd for $\text{C}_{16}\text{H}_{36}\text{NO}_2$ ($\text{M} + \text{H}^+$): 274.2741. Found 274.2743. Anal. Calcd for $\text{C}_{16}\text{H}_{35}\text{NO}_2$: C 70.28, H 12.90, N 5.12. Found: C 69.65, H 12.91, N 5.04.



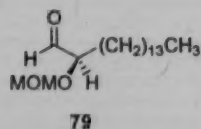
(2*S*,3*R*)-2-Aminooctadecane-1,3-diol (67b)

The title compound was prepared from **74b** according to the general procedure **D** using LiAlH_4 as reducing reagent. Compound **67b** was obtained as white solid by chromatography on silica gel using $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_4\text{OH}$ (100:10:1) as eluent in 78% yield: $R_f = 0.41$ (100:20:2 CH_2Cl_2 – MeOH – NH_4OH); $[\alpha]_D +8.1$ (c 1.0, MeOH). lit.^[160] $[\alpha]_D +1.83$ (c 1.0, pyridine), lit.^[142a] $[\alpha]_D +5.7$ (c 2.74, CHCl_3 – MeOH 4:1); ^1H NMR (CD_3OD , 600 MHz): δ 3.72 (dd, 1H, $J_{1a,1b} = 10.9$ Hz, $J_{1a,2} = 4.2$ Hz, H-1a), 3.49 (ddd, 1H, $J_{3,4a} = 8.0$ Hz, $J_{3,4b} = 3.0$ Hz, H-3), 3.46 (dd, 1H, $J_{1b,2} = 7.6$ Hz, H-1b), 2.70 (ddd, 1H, $J_{2,3} = 5.4$ Hz, H-2), 1.52 (m, 2H), 1.25–1.45 (m, 26H), 0.9 (t, 3H, $J = 7.0$ Hz); ^{13}C NMR (CD_3OD , 125 MHz): δ 74.1, 64.4, 58.1, 34.4, 33.1, 30.1–30.7 (10C), 27.0, 23.7, 14.4. HRMS m/z Calcd for $\text{C}_{18}\text{H}_{40}\text{NO}_2$ ($\text{M} + \text{H}^+$): 302.3054. Found 302.3055. Anal. Calcd for $\text{C}_{18}\text{H}_{39}\text{NO}_2$: C 71.70, H 13.04, N 4.65. Found: C 71.46, H 12.95, N 4.58.



(2*S*,3*R*)-2-Aminoicosane-1,3-diol (67c)

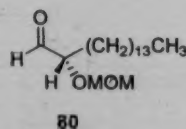
The title compound was prepared from **74c** according to the general procedure **D** using LiAlH_4 as reducing reagent. Compound **67c** was obtained as white solid by chromatography on silica gel using an eluent of $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_4\text{OH}$ (100:10:1) in 81% yield: $R_f = 0.19$ (100:10:2 CH_2Cl_2 -MeOH- NH_4OH); $[\alpha]_D +6.5$ (c 0.9, CHCl_3 -MeOH 4:1); ^1H NMR (CD_3OD , 600 MHz): δ 3.72 (dd, 1H, $J_{1a,1b} = 10.9$ Hz, $J_{1a,2} = 4.2$ Hz, H-1a), 3.49 (ddd, 1H, $J_{3,4a} = 8.0$ Hz, $J_{3,4b} = 3.0$ Hz, H-3), 3.46 (dd, 1H, $J_{1b,2} = 7.6$ Hz, H-1b), 2.70 (ddd, 1H, $J_{2,3} = 5.4$ Hz, H-2), 1.53 (m, 2H), 1.25–1.44 (m, 30H), 0.9 (t, 3H, $J = 7.0$ Hz); ^{13}C NMR (CD_3OD , 125 MHz): δ 74.1, 64.4, 58.1, 34.4, 33.1, 30.8–30.5 (12C), 27.4, 23.7, 14.4. HRMS m/z Calcd for $\text{C}_{20}\text{H}_{44}\text{NO}_2$ ($\text{M}+\text{H}^+$): 330.3366. Found 330.3363. Anal. Calcd for $\text{C}_{20}\text{H}_{43}\text{NO}_2$: C 72.89, H 13.15, N 4.25. Found: C 72.56, H 13.04, N 4.16.

**(2*R*)-2-Methoxymethoxyhexadecanal (79)**

The title compound was prepared from **77** according to the general procedure **E**. Compound **79** was obtained by chromatography on silica gel using an eluent of hexane/EtOAc (20:1) as a white solid in 90% yield: $R_f = 0.51$ (6:1 hexane-EtOAc); $[\alpha]_D +24.7$ (c 1.0, CHCl_3); ^1H NMR (CD_2Cl_2 , 500 MHz): δ 9.58 (d, 1H, $J = 2.0$ Hz), 4.70 (d, 1H, $J = 7.0$ Hz), 4.68 (d, 1H, $J = 7.0$ Hz), 3.85 (ddd, 1H, $J = 6.6$ Hz, 5.6 Hz, 2.0 Hz), 3.39 (s, 3H), 1.64 (m, 2H), 1.41 (m, 2H), 1.20–1.35 (m, 22H), 0.89 (t, 3H, $J = 7.0$ Hz); ^{13}C NMR (CD_2Cl_2 , 125 MHz): δ 203.3, 97.1, 82.9, 56.1, 32.3, 30.4, 30.1–29.7 (9C), 25.2,

23.1, 14.3. HRMS m/z Calcd for $C_{18}H_{36}O_3Na$ ($M + Na^+$): 323.2562. Found: 323.2564.

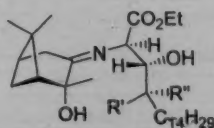
Anal. Calcd for $C_{18}H_{36}O_3$: C 71.95, H 12.08. Found: C 71.83, H 12.19.



(2S)-2-Methoxymethoxyhexadecanal (80)

The title compound was prepared from **78** according to the general procedure **E**.

Compound **80** was obtained by chromatography on silica gel using an eluent of hexane/EtOAc (20:1) as a white solid in 94% yield: R_f = 0.50 (6:1 hexane–EtOAc); $[\alpha]_D - 24.3$ (c 0.9, $CHCl_3$); ^1H NMR (CD_2Cl_2 , 500 MHz): δ 9.58 (d, 1H, J = 2.0 Hz), 4.70 (d, 1H, J = 7.0 Hz), 4.68 (d, 1H, J = 7.0 Hz), 3.86 (ddd, 1H, J = 7.6 Hz, 5.6 Hz, 2.2 Hz), 3.39 (s, 3H), 1.62–1.68 (m, 2H), 1.40 (m, 2H), 1.25–1.35 (m, 22H), 0.89 (t, 3H, J = 7.0 Hz); ^{13}C NMR (CD_2Cl_2 , 125 MHz): δ 203.3, 97.1, 82.8, 56.1, 32.3, 30.4, 30.1–29.7 (9C), 25.2, 23.1, 14.3. HRMS m/z Calcd for $C_{18}H_{36}O_3Na$ ($M + Na^+$): 323.2562. Found: 323.2561. Anal. Calcd for $C_{18}H_{36}O_3$: C 71.95, H 12.08. Found: C 71.62, H 12.14.

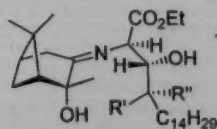


81 R' =OMOM, R'' =H

Ethyl {1R-[1 α ,2 β ,3(2R,3S,4R),5 α]}-3,4-dihydroxy-2-[(2-hydroxy-2,6,6-trimethylbicyclo[3,1,1]hept-3-ylidene)amino]octadecanoate (81)

The title compound was prepared from **79** according to the general procedure **B** using $ClTi(OEt)_3$ as reagent. Compound **81** (containing trace amounts of isopropyl ester) was

obtained as a single diastereoisomer by chromatography on silica gel using hexane/EtOAc (6:1) in 91% yield: $R_f = 0.31$ (3:1 hexane–EtOAc); ^1H NMR (CDCl_3 , 600 MHz): δ 4.78 (d, 1H, $J = 6.7$ Hz), 4.58 (d, 1H, $J = 6.8$ Hz), 4.24–4.30 (m, 2H), 4.17 (dt, 2H, $J = 7.1$ Hz, 0.9 Hz), 3.67 (dt, 1H, $J = 8.5$ Hz, 3.2 Hz), 3.41 (s, 3H), 3.23 (br s, 1H), 2.58 (broad s, 2H), 2.32 (ddd, 1H, $J = 10.7$ Hz, 6.0 Hz, 5.8 Hz), 2.06 (dd, 1H, $J = 6.0$ Hz, 5.9 Hz), 2.02 (ddd, 1H, $J = 6.0$ Hz, 5.9 Hz, 3.0 Hz), 1.58–1.70 (m, 2H), 1.52 (d, 1H, $J = 10.7$ Hz), 1.47 (s, 3H), 1.33 (s, 3H), 1.25 (t, 3H, $J = 7$ Hz), 1.25–1.30 (m, 24H), 0.88 (t, 3H, $J = 7.0$ Hz), 0.87 (s, 3H); ^{13}C NMR (CDCl_3 , 125 MHz): δ 180.3, 170.2, 96.4, 78.9, 76.7, 73.8, 64.6, 61.1, 55.8, 50.2, 38.6, 38.4, 34.1, 31.9, 30.2, 29.8–29.3 (7C), 28.3, 28.0, 27.3, 25.4, 22.8, 22.7, 14.2, 14.1. HRMS m/z Calcd for $\text{C}_{32}\text{H}_{60}\text{NO}_6$ ($\text{M} + \text{H}^+$): 554.4421. Found 554.4423.

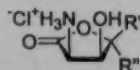


82 $\text{R}' = \text{H}$, $\text{R}'' = \text{OMOM}$

Ethyl {1*R*-[1 α ,2 β ,3(2*R*,3*S*,4*S*),5 α]}-3,4-dihydroxy-2-[(2-hydroxy-2,6,6-trimethylbicyclo [3,1,1] hept-3-ylidene)amino]octadecanoate (82)

The title compound was prepared from **80** according to the general procedure **B** using $\text{ClTi}(\text{OEt})_3$ as reagent. Compound **82** (containing trace amounts of isopropyl ester) was obtained as a single diastereoisomer by chromatography on silica gel using hexane/EtOAc (6:1) in 83% yield: $R_f = 0.32$ (hexane–EtOAc 3:1); ^1H NMR (CDCl_3 , 600 MHz): δ 4.66 (d, 1H, $J = 6.4$ Hz), 4.63 (d, 1H, $J = 6.4$ Hz), 4.31 (d, 1H, $J = 8.2$ Hz), 4.20 (quint, 2H, $J = 7.0$ Hz), 4.12 (quint, 1H, $J = 7.1$ Hz), 3.57 (ddd, 1H, $J = 8$ Hz, 5.9 Hz, 2

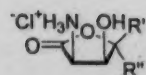
Hz), 3.37 (s, 3H), 3.01 (broad s, 1H), 2.66 (dd, 1H, $J = 8.2$ Hz, 2.8 Hz), 2.58 (ddd, 1H, $J = 8.1$ Hz, 2.5 Hz, 2.5 Hz), 2.34 (dddd, 1H, $J = 10.7$ Hz, 6.0 Hz, 5.8 Hz, 2.5 Hz), 2.08 (dd, 1H, $J = 5.9$ Hz, 5.9 Hz), 2.03 (ddd, 1H, $J = 6.0$ Hz, 5.9 Hz, 2.9 Hz), 1.74 (m, 1H), 1.64 (m, 1H), 1.52 (d, 1H, $J = 10.7$ Hz), 1.48 (s, 3H), 1.32 (s, 3H), 1.24 (t, 3H, $J = 7.0$ Hz), 1.25–1.30 (m, 24H), 0.88 (t, 3H, $J = 7.0$ Hz), 0.85 (s, 3H); ^{13}C NMR (CDCl_3 , 125 MHz): δ 180.5, 171.0, 96.5, 77.9, 76.7, 73.4, 65.0, 61.2, 60.4, 55.9, 50.2, 38.6, 38.4, 34.0, 31.9, 31.1, 29.8–29.3 (8C), 28.3, 28.0, 27.3, 25.6, 22.8, 22.7, 14.2, 14.1. HRMS m/z Calcd for $\text{C}_{32}\text{H}_{59}\text{NO}_6\text{Na}$ ($\text{M} + \text{Na}^+$): 576.4240. Found 576.4239.



85 $\text{R}' = \text{H}$, $\text{R}'' = \text{C}_{14}\text{H}_{29}$

(2R,3S,4R)-2-Amino-3-hydroxyoctadecane-1,4-lactone hydrochloride (85)

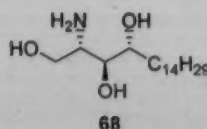
The title compound was prepared from **81** according to the general procedure C. Compound **85** was obtained as a single diastereoisomer by chromatography on silica gel using $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_4\text{OH}$ (100:2.5:1) as eluent in 79% yield: $R_f = 0.27$ (CH_2Cl_2 – MeOH – NH_4OH 100:5:2); ^1H NMR (CD_3OD , 600 MHz): δ 4.47 (d, 1H, $J = 6.2$ Hz), 4.45 (d, 1H, $J = 7.4$ Hz), 4.36 (d, 1H, $J = 5.3$ Hz), 1.8 (quint, 2H, $J = 7.5$ Hz), 1.24–1.50 (m, 24H), 0.89 (t, 3H, $J = 7.0$ Hz). ^{13}C NMR (CD_3OD , 125 MHz): δ 172.7, 89.8, 70.5, 52.1, 33.1, 32.9, 30.8, 30.7, 30.7, 30.7, 30.7, 30.6, 30.3, 26.6, 23.7, 14.4. HRMS m/z Calcd for $\text{C}_{18}\text{H}_{36}\text{NO}_3$ ($\text{M} + \text{H}^+$): 314.2690. Found 314.2691. Anal. Calcd for $\text{C}_{18}\text{H}_{35}\text{NO}_3$: C 68.97, H 11.25, N 4.47. Found: C 68.69, H 11.36, N 4.43.



86 $R' = C_{14}H_{29}$, $R'' = H$

(2*R*,3*S*,4*S*)-2-Amino-3-hydroxyoctadecane-1,4-lactone hydrochloride (86)

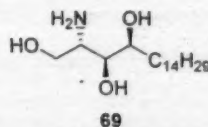
The title compound was prepared from **82** according to the general procedure **C**. Compound **86** was obtained as a single diastereoisomer by chromatography on silica gel using the eluent of $CH_2Cl_2/CH_3OH/NH_4OH$ (100:2.5:1) in 83% yield: $R_f = 0.26$ (CH_2Cl_2 - $MeOH-NH_4OH$ 100:5:2); ^1H NMR (CD_3OD , 600 MHz): δ 4.52 (ddd, 1H, $J = 5.2$ Hz, 2.6 Hz, 2.6 Hz), 4.50 (dd, 1H, $J = 5.2$ Hz, 5.0 Hz), 4.43 (d, 1H, $J = 5.0$ Hz), 1.82 (m, 1H), 1.72 (m, 1H), 1.48 (m, 2H), 1.25–1.40 (m, 22H), 0.89 (t, 3H, $J = 7.0$ Hz); ^{13}C NMR (CD_3OD , 125 MHz): δ 172.9, 84.9, 69.5, 54.4, 33.1, 30.9, 30.8, 30.8, 30.7, 30.7, 30.6, 30.6, 30.6, 30.5, 29.3, 26.3, 23.7, 14.4. HRMS m/z Calcd for $C_{18}H_{36}NO_3$ ($M + H^+$): 314.2690. Found 314.2694. Anal. Calcd for $C_{18}H_{35}NO_3HCl$: C 61.78, H 10.37, N 4.00. Found: C 61.50, H 10.66, N 3.74.



(2*S*,3*S*,4*R*)-2-Aminooctadecane-1,3,4-triol (D-ribo-phytosphingosine) (68)

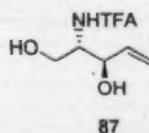
The title compound was prepared from **85** according to the general procedure **D** using $LiAlH_4$ as reducing reagent. Compound **68** was obtained as white solid by chromatography on silica gel using $CH_2Cl_2/CH_3OH/NH_4OH$ (100:10:1) as eluent in 82% yield: $R_f = 0.13$ (CH_2Cl_2 - $MeOH-NH_4OH$ 100:10:2); $[\alpha]_D +7.6$ (c 0.7, pyridine); {lit.^[162] $[\alpha]_D +8.7$ (c 0.8, pyridine); lit.^[163] $[\alpha]_D +8.5$ (c 0.7, pyridine);} ^1H NMR (CD_3OD , 500 MHz): δ 3.75 (dd, 1H, $J_{1a,1b} = 10.9$ Hz, $J_{1a,2} = 4.2$ Hz, H-1a), 3.56 (dd, 1H, $J_{1b,2} = 6.6$ Hz,

H-1b), 3.51 (ddd, 1H, $J_{4,5a} = 8.3$ Hz, $J_{4,5b} = 3.0$ Hz, H-4), 3.33 (dd, 1H, $J_{3,4} = 7.8$ Hz, H-3), 2.94 (ddd, 1H, $J_{2,3} = 5.7$ Hz, H-2), 1.73 (m, 1H), 1.55 (m, 1H), 1.25–1.40 (m, 24H), 0.9 (t, 3H, $J = 7.0$ Hz); ^{13}C NMR (CD_3OD , 125 MHz): δ 76.5, 74.5, 64.2, 55.7, 34.8, 33.1, 30.9–30.5 (9C), 26.6, 23.7, 14.4. HRMS m/z Calcd for $\text{C}_{18}\text{H}_{40}\text{NO}_3$ ($\text{M}+\text{H}^+$) 318.3002. Found 318.3001. Anal. Calcd for $\text{C}_{18}\text{H}_{39}\text{NO}_3$: C 68.09, H 12.38, N 4.41. Found: C 67.45, H 12.53, N 4.29.



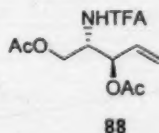
(2S,3S,4S)-2-Aminooctadecane-1,3,4-triol (L-lyxo-phytosphingosine) (69)

The title compound was prepared from **86** according to the general procedure **D** using LiAlH_4 as reducing reagent. Compound **69** was obtained as a white solid by chromatography on silica gel using $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}/\text{NH}_4\text{OH}$ (100:10:1) as eluent in 80% yield: $R_f = 0.14$ ($\text{CH}_2\text{Cl}_2\text{--MeOH--NH}_4\text{OH}$ 100:10:2); $[\alpha]_D -10.0$ (c 1.0, pyridine); {lit.^[142a] $[\alpha]_D -7.4$ (c 0.9, pyridine); lit.^[163] $[\alpha]_D -6.2$ (c 1.0, pyridine);} ^1H NMR (CD_3OD , 600 MHz): δ 3.76 (dd, 1H, $J_{1a,1b} = 10.9$ Hz, $J_{1a,2} = 4.2$ Hz, H-1a), 3.67 (ddd, 1H, $J_{4,5a} = 7.7$ Hz, $J_{4,5b} = 4.8$ Hz, H-4), 3.52 (dd, 1H, $J_{1b,2} = 7.0$ Hz, H-1b), 3.32 (dd, 1H, $J_{3,4} = 2.7$ Hz, H-3), 2.93 (ddd, 1H, $J_{2,3} = 7.0$ Hz, H-2), 1.45–1.59 (m, 2H), 1.25–1.40 (m, 24H), 0.9 (t, 3H, $J = 7.0$ Hz); ^{13}C NMR (CD_3OD , 125 MHz): δ 75.3, 72.3, 64.7, 55.6, 34.6, 33.1, 30.9–30.5 (9C), 27.0, 23.7, 14.4. HRMS m/z Calcd for $\text{C}_{18}\text{H}_{40}\text{NO}_3$ ($\text{M}+\text{H}^+$): 318.3002. Found 318.3002. Anal. Calcd for $\text{C}_{18}\text{H}_{39}\text{NO}_3$: C 68.09, H 12.38, N 4.41. Found: C 67.70, H 12.57, N 4.33.



(2*S*, 3*R*)-1,3-Di-*O*-acetyl-2-*N*-trifluoroacetamido-(4*E*)-octadecene-1,3-diol (87)

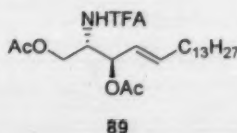
Compound **66** (160 mg, 1.37 mmol) was dissolved in dry methanol (10 mL) and then methyl trifluoroacetate (355 mg, 0.28 mmol) and triethylamine (0.2 mL, 1.4 mmol) were added. The mixture was stirred at room temperature for 18 h. Next the mixture was concentrated under reduced pressure and purified by chromatography on silica gel (3% methanol in dichloromethane) to afford the desired compound **87** as a white solid (221 mg, 76%): $R_f = 0.27$ (20:1 CH_2Cl_2 - CH_3OH); $[\alpha]_D -25.4$ (c 1.46, CH_3OH); ^1H NMR (500 MHz, CD_3OD) δ 5.84 (ddd, 1H, $J_{4,5t} = 17.1$ Hz, $J_{4,5c} = 10.4$ Hz, H-4), 5.28 (ddd, 1H, $J_{5t,5c} = 1.6$ Hz, H-5t), 5.17 (ddd, 1H, H-5c), 4.14 (ddd, 1H, $J_{3,4} = 7.3$ Hz, $J_{3,5c} = J_{3,5t} = 1.2$ Hz, H-3), 3.93 (ddd, 1H, $J_{2,3} = 7.2$ Hz, H-2), 3.80 (dd, 1H, $J_{1a,1b} = 11.5$ Hz, $J_{1a,2} = 3.9$ Hz, H-1a), 3.72 (dd, 1H, $J_{1b,2} = 7.2$ Hz, H-1b); ^{13}C NMR (125 MHz, CDCl_3) δ 159.1 (q, $J_{\text{C,F}} = 37$ Hz), 139.1, 117.6 (q, $J_{\text{C,F}} = 287$ Hz), 117.3, 73.3, 61.4, 57.6; HRMS m/z calcd for $\text{C}_{17}\text{H}_{31}\text{NO}_5$: 336.0505. Found: 336.0506.



(2*S*, 3*R*)-1,3-Di-*O*-acetyl-2-*N*-trifluoroacetamido-(4*E*)-octadecene-1,3-diol (88)

Crude **87** (202 mg, 0.95 mmol) was dissolved in dry pyridine (5 mL) and acetic anhydride (0.18 mL, 1.9 mmol) was added. The mixture was stirred at RT for 18 h. The mixture was diluted in dichloromethane (20 mL) and the organic phase was washed by

sat. NaHCO_3 , water, brine and dried over anhydrous Na_2SO_4 . The solution was concentrated and purified by chromatography on silica gel to give the desired product as a colorless oil **88** (268 mg, 95%): $R_f = 0.25$ (5:1 hexane-ethyl acetate); $[\alpha]_D +1.99$ (c 1.55, CHCl_3); ^1H NMR (500 MHz, CDCl_3) δ 6.83 (d, 1H, $J_{\text{NH},2} = 8.4$ Hz, NH), 5.80 (ddd, 1H, $J_{4,5t} = 17.0$ Hz, $J_{4,5c} = 10.5$ Hz, $J_{3,4} = 7.0$ Hz, H-4), 5.30-5.43 (m, 3H, H-5t, H-5c, H-3), 4.48 (m, 1H, H-2), 4.24 (dd, 1H, $J_{1a,1b} = 11.9$ Hz, $J_{1a,2} = 6.8$ Hz, H-1a) 4.17 (dd, 1H, $J_{1b,2} = 3.9$ Hz, H-1b), 2.11 (s, 3H, COCH_3), 2.09 (s, 3H, COCH_3); ^{13}C NMR (125 MHz, CDCl_3) δ 170.9, 169.8, 157.2 (q, $J_{\text{C},\text{F}} = 37$ Hz), 131.4, 120.3, 116.8 (q, $J_{\text{C},\text{F}} = 288$ Hz), 73.1, 61.6, 51.4, 20.8, 20.6; HRMS m/z calcd for $\text{C}_{11}\text{H}_{14}\text{NO}_5\text{F}_3\text{Na}$: 320.0716. Found: 320.0715. Anal calcd for $\text{C}_{11}\text{H}_{14}\text{NO}_5\text{F}_3$: C 44.45, H 4.75, N 4.71. Found: C 44.27, H 4.63, N 4.75.

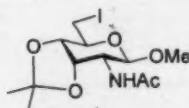


(2S, 3R)-1,3-Di-O-acetyl-2-N-trifluoroacetamido-(4E)-octadecene-1,3-diol (89)

Compound **88** (50 mg, 0.168 mmol) was dissolved in dry dichloromethane (5 mL) and 1-pentadecene (182 μL , 0.67 mmol) was added followed by the addition of 20 % Grubbs second generation catalyst (28.5 mg) in five portions (protocol B) at reflux. The mixture was gently refluxed under argon protection until almost all starting material has been consumed. The mixture was concentrated and purified by silica gel chromatography using hexane/ethyl acetate (2:1) as eluent to afford the desired *E*-isomer **89** as a white solid (59 mg, 74%): $R_f = 0.43$ (5:1 hexane-ethyl acetate); $[\alpha]_D -17.3$ (c 1.46, CHCl_3); ^1H NMR (500 MHz, CDCl_3) δ 6.62 (d, 1H, $J_{\text{NH},2} = 8.9$ Hz, NH), 5.86 (ddd, 1H, $J_{5,6a} = J_{5,6b} =$

6.8 Hz, H-5), 5.39 (dddd, 1H, $J_{4,5} = 15.2$ Hz, $J_{4,6a} = J_{4,6b} = 1.4$ Hz, H-4), 6.32 (dd, 1H, $J_{3,4} = 7.4$ Hz, H-3), 4.43 (dddd, 1H, $J_{2,3} = 6.1$ Hz, H-2), 4.33 (dd, 1H, $J_{1a,1b} = 11.9$ Hz, $J_{1a,2} = 6.6$ Hz, H-1a), 4.14 (dd, 1H, $J_{1b,2} = 3.8$ Hz, H-1b), 2.09 (s, 6H, 2x COCH₃), 2.04 (q, 2H, $J = 7.8$ Hz, alkane CH₂), 1.20-1.40 (m, 22H, alkane CH₂), 0.89 (t, 3H, $J = 6.8$ Hz, alkyl CH₃); ¹³C NMR (125MHz, CDCl₃) δ 170.9, 169.9, 138.5, 123.0, 73.2, 61.8, 51.6, 32.3, 31.9, 29.7, 29.6, 29.6, 29.5, 29.4, 29.3, 29.1, 28.7, 22.7, 21.0, 20.6, 14.1; HRMS *m/z* calcd for C₂₄H₄₀NO₅F₃Na: 502.2751. Found: 502.2754. Anal calcd for C₂₄H₄₀NO₅F₃: C 60.11, H 8.41, N 2.92. Found: C 60.24, H 8.32, N 3.09.

Chapter Five - Part I
Synthesis of α -Galactosylceramide (KRN7000)



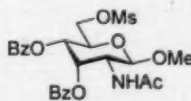
93

Methyl 2-acetamido-2,6-dideoxy-6-iodo-3,4-*O*-isopropylidene- β -D-glucopyranoside (93)

Methyl 2-acetamido-2-deoxy-6-*O*-methylsulfonyl- β -D-allopyranoside **53** (5 g, 14.5 mmol) was dissolved in a dry acetone (150 mL) and drierite (2 g) was added. To the mixture were added 2,2-dimethoxypropane (10 mL) and a catalytic amount of *p*-toluenesulfonic acid (50 mg). The mixture was stirred at 50 °C for 1 h (TLC showed that all starting material had been consumed). After concentration, the residue was dissolved in dichloromethane (100 mL) and this solution was washed with water, brine and dried over anhydrous Na₂SO₄. Filtration and concentration gave a crude residue **91**, which was used in the next step without further purification.

The residue **91** was redissolved in dry acetone (150 mL) and NaI (12 g) was added. The mixture was stirred under reflux for 18 h. After concentration, the residue was dissolved in dichloromethane (50 mL) and this solution was washed with water, brine and dried over anhydrous Na₂SO₄. Subsequent concentration and chromatography of the residue on silica gel using hexane/ethyl acetate (1:2) as eluent provided the target product **93** as a white solid in almost quantitative yield (5.5 g): $R_f = 0.58$ (20:1 CH₂Cl₂-CH₃OH); $[\alpha]_D -26.78$ (c 0.73, CHCl₃); ¹H NMR (500, CDCl₃) δ 5.76 (d, 1H, $J_{NH,H-2} = 9.0$ Hz, NH), 4.47 (d, 1H, $J_{1,2} = 8.4$ Hz, H-1), 4.40 (dd, 1H, $J_{3,4} = 4.6$ Hz, H-3), 4.35 (ddd, 1H, $J_{2,3} = 4.4$

Hz, H-2), 3.85 (dd, 1H, $J_{4,5}$ = 8.8 Hz, H-4), 3.54 (s, 3H, OCH₃), 3.49 (dd, 1H, $J_{6a,6b}$ = 10.8 Hz, H-6a), 3.38 (ddd, 1H, $J_{5,6b}$ = 8.5 Hz, $J_{5,6a}$ = 2.7 Hz, H-5), 3.19 (dd, 1H, H-6b), 2.04 (s, 3H, NHCOCH₃), 1.48 (s, 3H, C(CH₃)₂), 1.33 (s, 3H, C(CH₃)₂); ¹³C NMR (125 MHz, CDCl₃) δ 169.7, 110.4, 101.1, 75.7, 75.2, 56.7, 49.4, 28.2, 26.0, 23.5, 5.4; ESI HRMS m/z calcd for C₁₂H₂₀NO₅INa (M+Na⁺): 408.0279. Found: 408.0282. Anal Calcd for C₁₂H₂₀NO₅I: C 37.42, H 5.23, N 3.64. Found: C 37.72, H 5.18, N 3.68.

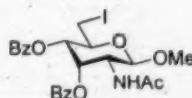


92

Methyl 2-acetamido-3,4-di-O-benzoyl-2-deoxy-6-O-methanesulfonyl- β -D-allopyranoside (92)

Benzoyl chloride (0.2 mL, 8 mmol) was added portionwise to compound **53** (1.2 g, 3.8 mmol) in dry pyridine (50 mL) at 0 °C. The mixture was stirred and allowed to warm from 0 °C to RT over 18 h. The reaction was quenched by addition of methanol. After concentration, the residue was dissolved in dichloromethane (20 mL) and the solution was extracted with water, washed by brine and dried with anhydrous Na₂SO₄. The filtrate was concentrated to give a residue, which was purified by silica gel chromatography to afford the desired compound **92** as a white solid (1.6 g, 77%): R_f = 0.30 (1:4 hexane-ethyl acetate); $[\alpha]_D$ -32.2 (c 1.42, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 8.01 (d, 2H, J = 7.0 Hz, ArH), 7.79 (d, 2H, J = 7.0 Hz, ArH), 7.66 (dd, 1H, J = 7.5 Hz, ArH), 7.52 (dd, 3H, J = 7.3 Hz, ArH), 7.32 (dd, 2H, J = 8.1 Hz, ArH), 6.00 (dd, 1H, $J_{3,4}$ = 3.0 Hz, H-3), 5.66 (d, 1H, $J_{NH,H-2}$ = 8.8 Hz, NH), 5.33 (dd, 1H, $J_{4,5}$ = 9.3 Hz, H-4), 4.83 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1), 4.50 (ddd, 1H, $J_{2,3}$ = 3.0 Hz, H-2), 4.46 (dd, 1H, $J_{6a,6b}$ = 10.8 Hz, H-6a), 4.40 (ddd,

$J_{5,6a} = 2.3$ Hz, $J_{5,6b} = 5.5$ Hz, H-5), 4.37 (dd, 1H, H-6b), 3.58 (s, 3H, SO_2CH_3), 3.05 (s, 3H, OCH_3), 1.96 (s, 3H, NHCOCH_3); ^{13}C NMR (125 MHz, CDCl_3) δ 169.7, 165.1, 164.7, 133.9, 133.5, 129.7, 129.7, 129.1, 128.8, 128.7, 128.4, 100.7, 71.0, 70.0, 67.9, 67.4, 56.7, 50.7, 37.7, 23.2; ESI HRMS m/z Calcd for $\text{C}_{24}\text{H}_{27}\text{NO}_{10}\text{SNa}$ ($\text{M}+\text{Na}^+$): 544.1248. Found: 544.1247.



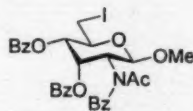
94

Methyl 2-acetamido-3,4-di-O-benzoyl-2,6-dideoxy-6-iodo- β -D-allopyranoside (94)

Crude **92** (1.04 g, 2 mmol) was dissolved in dry acetone (30 mL) and NaI (1.5 g, 10 mmol) was added. The mixture was stirred under reflux for 18 h and concentrated. The resulting residue was diluted with dichloromethane (20 mL), washed with water, brine and dried over anhydrous Na_2SO_4 . The removal of solvent gave a crude residue, which was purified by silica gel chromatography to afford the desired compound **94** as white solid (906 mg, 82%); $R_f = 0.29$ (1:4 hexane-ethyl acetate); $[\alpha]_D -56.3$ (c 0.3, CHCl_3); ^1H NMR (600 MHz, CDCl_3) δ 8.02 (dd, 2H, $J = 8.1$ Hz, $J = 1.2$ Hz, ArH), 7.81 (dd, 2H, $J = 8.2$ Hz, $J = 1.2$ Hz, ArH), 7.67 (dd, 1H, $J = 7.5$ Hz, ArH), 7.52 (dd, 3H, $J = 7.9$ Hz, 7.9 Hz, ArH), 7.33 (dd, 2H, $J = 7.8$ Hz, 7.8 Hz, ArH), 5.97 (dd, 1H, $J_{3,4} = 3.1$ Hz, H-3), 5.61 (d, 1H, $J_{\text{NH},\text{H}-2} = 8.7$ Hz, NH), 5.23 (dd, 1H, $J_{4,5} = 9.2$ Hz, H-4), 4.82 (d, 1H, $J_{1,2} = 8.1$ Hz, H-1), 4.50 (ddd, 1H, $J_{2,3} = 3.1$ Hz, H-2), 4.10 (ddd, 1H, $J_{5,6b} = 8.8$ Hz, $J_{5,6a} = 3.1$ Hz, H-5), 3.63 (s, 3H, OCH_3), 3.42 (dd, 1H, $J_{6a,6b} = 11.0$ Hz, H-6a), 3.29 (dd, 1H, H-6b), 1.95 (s, 3H, NHCOCH_3); ^{13}C NMR (125 MHz, CDCl_3) δ 169.5, 165.1, 164.7, 133.8, 133.5, 129.7,

129.2, 128.9, 128.8, 128.7, 128.4, 100.7(C-1), 72.9, 71.2, 70.2, 56.6, 51.1, 23.3, 4.4 (C-6);

ESI HRMS m/z Calcd for $C_{23}H_{24}NO_7INa$ ($M+Na^+$): 576.0490. Found: 576.0489.

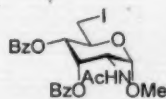


95b

Methyl 2-acetamido-3,4-di-*O*-benzoyl-2,6-dideoxy-6-iodo- β -D-allopyranoside (95b)

Benzoyl chloride (3 equiv, 0.24 mL, 1.8 mmol) was added portionwise to compound **53** (195 mg, 0.6 mmol) in dry pyridine (5 mL) at 0 °C. The mixture was stirred and allowed to warm from 0 °C to RT over 18 h. The reaction was quenched by addition of methanol. After concentration, the residue was dissolved in dichloromethane (50 mL) and the solution was extracted with water, washed by brine and dried with anhydrous Na_2SO_4 . The filtrate was concentrated to give a residue, which was separated by silica gel chromatography to give a major product **95a** and a dibenzoyl derivative as minor product. Next compound **95a** was dissolved in dry acetone (5 mL) and NaI (0.45 g, 3 mmol) was added. The mixture was stirred under reflux for 18 h and concentrated. The resulting residue was diluted with dichloromethane (5 mL), washed with water, brine and dried over anhydrous Na_2SO_4 . The removal of solvent gave a crude residue, which was purified by silica gel chromatography to afford the desired compound **95b** as white solid (232 mg, 59%). ^1H NMR (600 MHz, $CDCl_3$) δ 8.15 (dd, 2H, $J = 8.1$ Hz, $J = 1.2$ Hz, ArH), 7.86 (dd, 2H, $J = 8.2$ Hz, $J = 1.2$ Hz, ArH), 7.62 (dd, 3H, $J = 7.5$ Hz, ArH), 7.49 (dd, 4H, $J = 7.9$ Hz, 7.9 Hz, ArH), 7.32 (dd, 3H, $J = 7.8$ Hz, 7.8 Hz, ArH), 6.08 (dd, 1H, $J_{3,4} = 3.0$ Hz, H-3), 5.82 (d, 1H, $J_{1,2} = 8.4$ Hz, H-1), 5.24 (dd, 1H, $J_{4,5} = 9.9$ Hz, H-4), 4.91 (d, 1H, $J_{1,2} = 2.6$ Hz, H-2), 4.15 (m, 1H, H-5), 3.56 (s, 3H, OCH_3), 3.42 (dd, 1H, $J_{6a,6b} = 10.9$ Hz, H-6a),

3.28 (dd, 1H, $J_{6b,5} = 8.8$ Hz, H-6b), 2.04 (s, 3H, NHCOCH_3); ESI MS m/z Calcd for $\text{C}_{30}\text{H}_{28}\text{INO}_8\text{Na}$ ($\text{M}+\text{Na}^+$): 680.09. Found: 680.10.

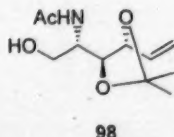


97b

Methyl 2-acetamido-3,4-di-*O*-benzoyl-2,6-dideoxy-6-iodo- α -D-allopyranoside (97b)

Benzoyl chloride (3 equiv, 2.7 mL, 21.8 mmol) was added portionwise to compound **96** (2.28 g, 7.28 mmol) in dry pyridine (60 mL) at 0 °C. The mixture was stirred and allowed to warm from 0 °C to RT over 18 h. The reaction was quenched by addition of methanol. After concentration, the residue was dissolved in dichloromethane (20 mL) and the solution was extracted with water, washed by brine and dried with anhydrous Na_2SO_4 . The filtrate was concentrated to give a residue **97a**, which was dissolved in dry acetone (50 mL) and NaI (6 g, 40 mmol) was added. The mixture was stirred under reflux for 18 h and concentrated. The resulting residue was diluted with dichloromethane (20 mL), washed with water, brine and dried over anhydrous Na_2SO_4 . The removal of solvent gave a crude residue, which was purified by silica gel chromatography to afford the desired compound **97b** as white solid (3.5 g, 87%). ^1H NMR (600 MHz, CDCl_3) δ 8.06 (dd, 2H, $J = 8.1$ Hz, $J = 1.2$ Hz, ArH), 7.80 (dd, 2H, $J = 8.2$ Hz, $J = 1.2$ Hz, ArH), 7.63 (dd, 1H, $J = 7.5$ Hz, ArH), 7.51 (dd, 3H, $J = 7.9$ Hz, 7.9 Hz, ArH), 7.32 (dd, 2H, $J = 7.8$ Hz, 7.8 Hz, ArH), 5.86 (dd, 1H, $J_{3,4} = 3.3$ Hz, H-3), 5.82 (d, 1H, $J_{\text{NH},\text{H}-2} = 9.3$ Hz, NH), 5.16 (dd, 1H, $J_{4,5} = 9.9$ Hz, H-4), 4.83 (d, 1H, $J_{1,2} = 4.3$ Hz, H-1), 4.68 (ddd, 1H, $J_{2,3} = 3.6$ Hz, H-2), 4.26 (ddd, 1H, $J_{5,6b} = 8.8$ Hz, $J_{5,6a} = 2.8$ Hz, H-5), 3.65 (s, 3H, OCH_3), 3.40 (dd, 1H, $J_{6a,6b} = 10.9$ Hz, H-6a), 3.23 (dd, 1H, H-6b), 1.95 (s, 3H, NHCOCH_3); ^{13}C NMR (125 MHz,

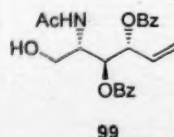
CDCl_3) δ 169.4, 164.7, 133.5, 133.4, 129.9, 129.7, 129.5, 128.6, 128.3, 98.1 (C-1), 70.9, 69.0, 65.4, 56.4, 48.2, 23.2, 4.8 (C-6).



(2*S*,3*S*,4*R*)-2-acetamido-3,4-*O*-isopropylidene-hex-5-ene-1,3,4-triol (98)

Compound **93** (1.2 g, 3.1 mmol) was dissolved in a mixture of THF/water (60 mL, 6:1, v/v) and freshly pre-active zinc powder (5 g) was added. The mixture was sonicated at 40–50 °C for 1 h (TLC showed all starting material had been consumed). The mixture was then passed through a short pad of silica using 8% methanol in dichloromethane as eluent. After concentration, the residue was dissolved in dry methanol (20 mL) and 5 equivalents of NaBH_4 (600 mg, 15 mmol) were added in portions at 0 °C. The mixture was stirred and allowed to warm from 0 °C to RT over 2 h. The reaction was quenched by addition of acetic acid (0.3 mL) at 0 °C. After concentration, the residue was chromatographed on silica gel using 4% methanol in dichloromethane to afford the desired product **98** as a white foam (0.66 g, 93%): R_f = 0.36 (10:1 CH_2Cl_2 - CH_3OH); $[\alpha]_D$ –19.9 (c 0.93, CHCl_3); ^1H NMR (500 MHz, CDCl_3) δ (d, 1H, $J_{\text{NH,H-2}}$ = 8.1 Hz, NH), 5.88 (ddd, 1H, $J_{5,6t}$ = 17.3 Hz, $J_{5,6c}$ = 10.4 Hz, H-5), 5.40 (ddd, 1H, $J_{6t,6c}$ = 1.4 Hz, H-6t), 5.30 (ddd, 1H, H-6c), 4.70 (dd, $J_{4,5}$ = 6.9 Hz, H-4), 4.30 (dd, 1H, $J_{3,4}$ = 6.4 Hz, H-3), 4.02 (dddd, 1H, $J_{2,3}$ = 5.9 Hz, H-2), 3.88 (dd, 1H, $J_{1a,1b}$ = 11.3 Hz, $J_{1a,2}$ = 3.3 Hz, H-1a), 3.68 (d, 1H, $J_{1b,2}$ = 3.5 Hz, H-1b), 2.64 (dd, 1H, $J_{\text{OH},1}$ = 7.4 Hz, 4.9 Hz, OH at C-1), 1.98 (s, 3H, NHCOCH_3), 1.50 (s, 3H, $\text{C}(\text{CH}_3)_2$), 1.36 (s, 3H, $\text{C}(\text{CH}_3)_2$); ^{13}C NMR (125 MHz, CDCl_3) δ 170.0, 133.0, 118.9, 108.9, 78.7, 78.5, 62.9, 50.5, 27.3, 24.9, 23.4; ESI HRMS

m/z calcd for $C_{11}H_{19}NO_4Na$ ($M+Na^+$): 252.1206. Found: 252.1207. Anal calcd for $C_{11}H_{19}NO_4$: C 57.62, H 8.35, N 6.11. Found: C 57.15, H 8.32, N 6.05.

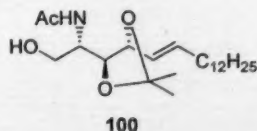


(2*S*,3*S*,4*R*)-2-acetamido-3,4-di-*O*-benzoyl-hex-5-ene-1,3,4-triol (99)

Compound **94** (500 mg, 0.9 mmol) was dissolved in a mixture of THF/water (10 mL, 4:1, v/v) and freshly pre-active zinc powder (1.5 g) was added. The mixture was stirred at 40–50 °C under sonication for 1 h (TLC showed all starting material had been consumed). The mixture was then passed through a short pad of silica by washing with 8% methanol in dichloromethane. After concentration, the residue was dissolved in dry methanol (15 mL) at 0 °C followed by addition of $NaBH_4$ (172 mg, 4.5 mmol) in portions. The mixture was stirred and allowed to warm from 0 °C to RT over 2 h. The reaction was quenched by addition of acetic acid (0.5 mL). Concentration and chromatography on silica using 4% methanol in dichloromethane afforded the desired product **99** as a white solid (253 mg, 71%): R_f = 0.24 (1:5 hexane-ethyl acetate); $[\alpha]_D^{20}$ +70.8 (c 1.7, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3/CD_3OD$ 4:1) δ 7.88 (d, 2H, J = 8.2 Hz, ArH), 7.81 (d, 2H, J = 8.1 Hz, ArH), 7.46 (t, 1H, J = 7.5 Hz, ArH), 7.40 (t, 1H, J = 7.4 Hz, ArH), 7.32 (t, 2H, J = 8.0 Hz, ArH), 7.25 (t, 2H, J = 8.2 Hz, ArH), 5.96 (ddd, 1H, $J_{5,6t}$ = 17.2 Hz, $J_{5,6c}$ = 10.6 Hz, $J_{5,4}$ = 6.1 Hz, H-5), 5.64 (m, 1H, H-4), 5.45 (ddd, 1H, $J_{3,4}$ = 3.1 Hz, H-3), 5.32 (dd, 1H, $J_{6t,6c}$ = 1.4 Hz, $J_{6t,4}$ = 1.4 Hz, H-6t), 5.26 (ddd, 1H, H-6c), 4.26 (ddd, 1H, $J_{2,3}$ = 9.3 Hz, H-2), 3.54 (dd, 1H, $J_{1a,1b}$ = 11.7 Hz, $J_{1a,2}$ = 3.3 Hz, H-1a), 3.50 (dd, 1H, $J_{1b,2}$ = 4.4 Hz, H-1b); 1.90 (s, 3H, $NHCOCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.7, 166.5, 166.0, 133.8,

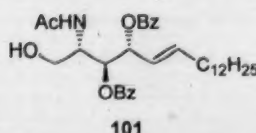
133.5, 131.1, 130.0, 129.9, 129.9, 129.5, 128.8, 128.6, 120.1, 74.7, 72.8, 61.0, 50.6, 22.9;

ESI HRMS m/z calcd for $(M+Na^+)$:420.1417. Found 420.1415.



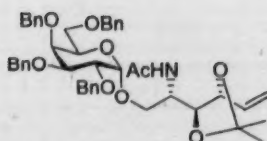
(2*S*,3*S*,4*R*)-2-Acetamido-3,4-*O*-isopropylidene-(5*E*)-octadecene-1,3,4-triol (100)

To a solution of compound **98** (60 mg, 0.26 mmol) and 1-tetradecene (0.33 mL, 1.3 mmol) in dry dichloromethane (9 mL), Grubbs' second generation catalyst (20%, 44 mg) was added in four portions (protocol B) under reflux. The reaction was continued for 18 h under gentle reflux (TLC showed that almost starting material had disappeared). Concentration followed by chromatography of the residue on silica using hexane/ethyl acetate (1:5) as eluent provided the target product **100** as a white solid (85 mg, 82%, *E/Z* = ~16:1); R_f = 0.22 (1:5 hexane-ethyl acetate); $[\alpha]_D -12.4$ (c 0.94, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 5.96 (d, 1H, $J_{NH,H-2}$ = 8.2 Hz, NH), 5.84 (dt, 1H, $J_{6,7}$ = 6.8 Hz, H-6), 5.50 (ddd, 1H, $J_{5,6}$ = 15.3 Hz, H-5), 4.67 (dd, 1H, $J_{4,5}$ = 7.3 Hz, H-4), 4.24 (dd, 1H, $J_{3,4}$ = 6.0 Hz, H-3), 4.03 (dddd, 1H, $J_{2,3}$ = 6.3 Hz, H-2), 3.90 (dd, 1H, $J_{1a,1b}$ = 11.5 Hz, $J_{1a,2}$ = 3.5 Hz, H-1a) 3.68 (dd, 1H, $J_{1b,2}$ = 3.5 Hz, H-1b), 2.02-2.08 (m, 2H, H₇ or =CHCH₂), 1.98 (s, 3H, NHCOCH₃), 1.50 (s, 3H, C(CH₃)₂), 1.36 (s, 3H, C(CH₃)₂), 1.20-1.40 (m, 20H, alkane CH₂), 0.88 (t, 3H, J = 6.8 Hz, alkyl CH₃); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.8 (C=O), 139.1, 124.2, 108.5, 78.8, 63.1, 50.7, 32.4, 31.9, 29.7, 29.6, 29.6, 29.6, 29.5, 29.3, 29.3, 29.0, 27.3, 24.8, 23.4, 22.7, 14.1; ESI HRMS m/z calcd for $C_{23}H_{43}NO_4Na$ ($M+Na^+$): 420.3084. Found 420.3085.



(2*S*,3*S*,4*R*)-2-acetamido-3,4-di-*O*-benzoyl-(5*E*)-octadecene-1,3,4-triol (101)

To a solution of compound **99** (50 mg, 0.126 mmol) and 1-tetradecene (0.16 mL, 0.63 mmol) in dry dichloromethane (5 mL), Grubbs' second generation catalyst (20%, 26.7 mg) was added in four portions (protocol B) under reflux. The reaction was continued under gentle reflux for 18 h (TLC showed that almost starting material has gone). Concentration and chromatography of the residue on silica gel using hexane/ethyl acetate (1:5) as eluent gave the target product **101** as a white solid (65 mg, 91%, *E/Z* = ~ 14:1): R_f = 0.48 (1:5 hexane-ethyl acetate); $[\alpha]_D^{+30.8}$ (c 0.48, CHCl_3); ^1H NMR (600 MHz, CDCl_3) δ 8.07 (d, 2H, J = 7.2 Hz, ArH), 7.96 (d, 2H, J = 10.8 Hz, ArH), 7.64 (t, 1H, J = 7.4 Hz, ArH), 7.48-7.54 (m, 3H, ArH), 7.38 (t, 2H, J = 7.7 Hz, ArH), 6.30 (d, 1H, $J_{\text{NH,H-2}}$ = 9.2 Hz, NH), 6.03 (ddd, 1H, $J_{5,6}$ = 14.3 Hz, $J_{6,7}$ = 6.9 Hz, 7.4 Hz, H-6), 5.74-5.82 (m, 2H, H-5 and H-4), 5.41 (dd, 1H, J = 9.1 Hz, 2.5 Hz, H-3), 4.39 (m, 1H, H-2), 3.69 (dd, 1H, $J_{1a,1b}$ = 12.3 Hz, $J_{1a,2}$ = 2.5 Hz, H-1a), 3.64 (dd, 1H, $J_{1b,2}$ = 2.9 Hz, H-1b), 2.15 (q, 2H, J = 7.2 Hz, H₇ or =CHCH₂), 2.1 (s, 3H, NHCOCH_3), 1.38-1.44 (m, 2H, alkane CH₂), 1.20-1.32 (m, 18H, alkane CH₂), 0.88 (t, 3H, J = 7.1 Hz, alkyl CH₃); ^{13}C NMR (125 MHz, CDCl_3) δ 170.0, 167.2, 165.5, 138.6, 133.9, 133.1, 130.0, 129.9, 129.7, 129.7, 129.0, 128.7, 128.6, 128.4, 122.0, 61.4, 50.3, 32.6, 31.9, 29.7, 29.6, 29.6, 29.4, 29.3, 29.3, 29.2, 28.9, 23.5, 22.7, 14.1; ESI HRMS m/z calcd for $\text{C}_{34}\text{H}_{47}\text{NO}_6\text{Na}$: 588.3296. Found 588.3297.

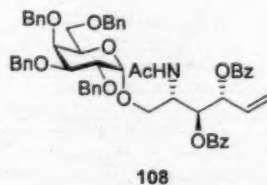


107

(2*S*,3*S*,4*R*)-3,4-*O*-Isopropylidene-1-*O*-(2,3,4,6-tetra-*O*-benzyl- α -D-galactopyranosyl)-2-acetamido-hex-5-ene-1,3,4-triol (107)

To a solution of 1-*O*-acetyl-2,3,4,6-tetra-*O*-benzyl-D-galactopyranoside **104** (290 mg, 0.50 mmol) in dry dichloromethane (3 mL) was added iodotrimethylsilane (100 mg, 0.5 mmol) at 0 °C. The mixture was stirred for 0 °C for 20 min and the reaction was stopped by adding dry toluene (3 mL), co-evaporating three times with dry toluene to give a pale yellow residue, which was dissolved in dry benzene (1 mL) under argon. Next in a separate flask, tetrabutylammonium iodide (549 mg, 1.49 mmol), acceptor **98** (38 mg, 0.166 mmol), DIPEA (64 mg, 0.5 mmol) and 4Å molecular sieves were added to a benzene solution (2 mL). This mixture was stirred under argon at 65 °C for 10 min, followed by addition of freshly prepared galactosyl iodide in benzene. The resulting mixture was stirred at 65 °C for 1-2 h. The reaction was stopped by addition of ethyl acetate (10 mL) and the mixture was cooled to 0 °C for precipitation of tetrabutylammonium iodide. The white precipitate and molecular sieves were filtered off. Concentration and subsequent chromatography of the residue on silica gel using 3:2 hexane/ethyl acetate as eluent afforded exclusively the desired α -anomer **107** as a white foam (111 mg, 89%); R_f = 0.45 (1:2 hexane-ethyl acetate); $[\alpha]_D^{+50}$ (c 1.29, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 7.24-7.42 (m, 20H, ArH), 6.33 (d, 1H, $J_{NH, H-2}$ = 9.3 Hz, NH), 5.72 (ddd, 1H, $J_{5,6t}$ = 17.0 Hz, $J_{5,6c}$ = 10.4 Hz, $J_{5,4}$ = 7.3 Hz, H-5_{sph}), 5.18 (ddd, 1H, $J_{6t,6c}$ = 1.4 Hz, H-6_t_{sph}), 5.13 (ddd, 1H, H-6_c_{sph}), 4.94 (d, 1H J = 11.6 Hz, PhCH₂), 4.87 (d, 1H,

$J_{1,2} = 3.8$ Hz, H-1), 4.82 (dd, 2H, $J = 11.6$ Hz, PhCH₂), 4.75 (d, 1H, $J = 11.7$ Hz, PhCH₂), 4.66 (d, 1H, $J = 11.6$ Hz, PhCH₂), 4.59 (d, 1H, $J = 11.6$ Hz, PhCH₂), 4.48 (d, 1H, $J = 11.7$ Hz, PhCH₂), 4.38 (m, 2H, PhCH₂ and H-4_{sph}), 4.24 (dd, 1H, $J = 9.7$ Hz, $J = 6.1$ Hz, H-3_{sph}), 4.11 (dd, 1H, $J_{1a,1b} = 11.7$ Hz, $J_{1a,2} = 3.0$ Hz, H-1a_{sph}), 4.05 (ddd, 1H, $J_{2,3} = 9.9$ Hz, H-2), 3.99 (m, 2H, H-2_{sph} and H-5), 3.92 (m, 2H, H-3 and H-4), 3.61 (dd, 1H, $J_{1b,2} = 2.6$ Hz, H-1b_{sph}), 3.56 (dd, 1H, $J_{6a,6b} = 9.5$ Hz, H-6a), 3.34 (dd, 1H, H-6b), 1.80 (s, 3H, NHCOCH₃), 1.46, 1.34 (s, 3H, C(CH₃)₂); ¹³C NMR (125 MHz, CDCl₃) δ 169.3 (C=O), 138.7, 138.4, 138.3, 133.2, 128.5, 128.4, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.6, 127.5, 118.0, 108.7, 100.1, 78.9, 78.8, 76.0, 74.7, 74.6, 73.7, 73.5, 73.1, 70.9, 70.0, 69.8, 49.3, 27.8, 25.6, 23.3; ESI HRMS m/z calcd for C₄₅H₅₃NO₉Na (M+Na⁺): 774.3612. Found 774.3612.

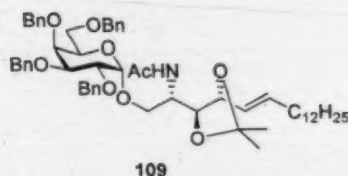


(2*S*,3*S*,4*R*)-3,4-Di-*O*-benzoyl-1-*O*-(2,3,4,6-tetra-*O*-benzyl- α -D-galactopyranosyl)-2-acetamido-hex-5-ene-1,3,4-triol (108)

Method A: Compound **99** (48 mg, 0.12 mmol) and tetrabenzyl galactosyl imidate **100** (164 mg, 0.24 mmol) were dissolved in a mixture of dry Et₂O (2 mL) and THF (0.2 mL) containing 4Å molecular sieves. The mixture was cooled to -50 °C, followed by addition of a catalytic amount of trimethylsilane triflate (2 μ L). The mixture was stirred at -50 °C for 1 h and warmed to -20 °C for another 1 h before slowly warming to RT. Next the solution was diluted with ethyl acetate and molecular sieves were filtered off. The

organic layer was washed with saturated NaHCO_3 , brine and dried over anhydrous Na_2SO_4 . Concentration and column chromatography of the residue with 2:1 hexane/ethyl acetate provided the desired α -anomer **108** as a colorless syrup (80 mg, 82%).

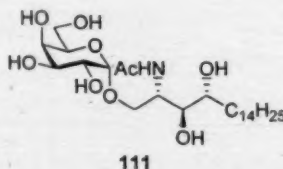
Method B: The desired product **108** was also prepared in 71% from the glycosidation of tetrabenzyl galactosyl iodide **105** with acceptor **99** according to the same experimental procedure as **107**: $R_f = 0.24$ (1:1 hexane-ethyl acetate); $[\alpha]_D +31.5$ (c 1.58, CHCl_3); ^1H NMR (600 MHz, CDCl_3) δ 8.06 (dd, 2H, $J = 8.1$ Hz, $J = 1.1$ Hz, ArH), 7.96 (dd, 2H, $J = 8.1$ Hz, $J = 1.2$ Hz, ArH), 7.61 (t, 1H, $J = 7.5$ Hz, ArH), 7.52 (t, 1H, $J = 7.5$ Hz, ArH), 7.47 (t, 2H, $J = 8.0$ Hz, ArH), 7.33-7.39 (m, 6H, ArH), 7.31 (m, 1H, ArH), 7.18-7.28 (m, 15H, ArH), 7.10 (d, 1H, $J_{\text{NH,H-2}} = 9.8$ Hz, NH), 6.06 (ddd, 1H, $J_{5,6t} = 17.3$ Hz, $J_{5,6c} = 10.7$ Hz, H-5_{sph}), 5.79 (dd, $J_{4,5} = 6.6$ Hz, H-4_{sph}), 5.76 (d, 1H, $J_{3,4} = 2.8$ Hz, H-3_{sph}), 5.45 (ddd, 1H, $J_{6t,6c} = 1.1$ Hz, H-6t), 5.41 (d, 1H, H-6c), 4.89 (d, 1H, $J = 11.5$ Hz, PhCH_2), 4.76 (d, 1H, $J = 11.5$ Hz, PhCH_2), 4.72 (d, 1H, $J_{1,2} = 3.8$ Hz, H-1), 4.70 (d, 1H, $J = 11.5$ Hz, PhCH_2), 4.68 (d, 1H, $J = 11.5$ Hz, PhCH_2), 4.63 (d, 1H, $J = 11.5$ Hz, PhCH_2), 4.55 (d, 1H, $J = 11.5$ Hz, PhCH_2), 4.52 (m, 2H, H-2_{sph} and PhCH_2), 4.41 (d, 1H, $J = 12.0$ Hz, PhCH_2), 4.22 (dd, 1H, $J_{5,6a} = 8.1$ Hz, $J_{5,6b} = 4.2$ Hz, H-5), 4.12 (dd, 1H, $J_{1a,1b} = 12.4$ Hz, $J_{1a,2} = 2.8$ Hz, H-1a_{sph}), 4.01 (dd, 1H, $J_{2,3} = 9.9$ Hz, H-2), 3.87 (m, 1H, H-3), 3.85 (d, 1H, $J = 2.9$ Hz, H-4), 3.57 (dd, 1H, $J_{1b,2} = 2.5$ Hz, H-1b_{sph}), 3.53 (dd, 1H, $J_{6a,6b} = 9.7$ Hz, H-6a), 3.22 (dd, 1H, H-6b), 1.89 (s, 3H, NHCOCH_3); ^{13}C NMR (125 MHz, CDCl_3) δ 170.0, 165.3, 165.2, 138.7, 138.3, 138.2, 137.3, 133.3, 133.0, 130.7, 129.9, 129.9, 129.8, 129.8, 128.6, 128.5, 128.4, 128.4, 128.3, 128.1, 128.0, 127.8, 127.8, 127.7, 127.5, 127.5, 120.4, 100.8, 78.7, 76.6, 74.8, 74.6, 74.5, 73.6, 73.4, 73.3, 72.1, 71.5, 70.6, 69.7, 48.9, 23.2; ESI HRMS m/z calcd for $\text{C}_{56}\text{H}_{57}\text{NO}_{11}\text{Na}$ ($\text{M} + \text{Na}^+$): 942.3824. Found: 942.3826.



(2*S*,3*S*,4*R*)-3,4-*O*-Isopropylidene-1-*O*-(2,3,4,6-tetra-*O*-benzyl- α -D-galactopyranosyl)-2-acetamido-(5*E*)-octadecene-1,3,4-triol (109)

To a solution of compound **107** (100 mg, 0.133 mmol) and 1-tetradecene (0.14 mL, 0.55 mmol) in dry dichloromethane (5 mL) was added Grubbs' second generation catalyst (20%, 22.6 mg) in four portions (protocol B) under reflux. The reaction was continued for 18 h under gentle reflux. Concentration and column chromatography of the residue using 3:1 hexane/ethyl acetate as eluent afforded the desired product **109** as a colorless syrup (112 mg, 91%): $R_f = 0.47$ (3:2 hexane-ethyl acetate); $[\alpha]_D +46.0$ (c 0.43, CHCl_3); ^1H NMR (500 MHz, CDCl_3) δ 7.24-7.42 (m, 20H, ArH), 6.24 (d, 1H, $J_{\text{NH,H-2}} = 9.4$ Hz, NH), 5.61 (dt, 1H, $J_{6,7} = 6.8$ Hz, H-6_{sph}), 5.39 (dd, 1H, $J_{5,6} = 15.3$ Hz, H-5_{sph}), 4.94 (d, 1H, $J = 11.5$ Hz, PhCH₂), 4.90 (d, 1H, $J_{1,2} = 3.8$ Hz, H-1), 4.84 (d, 1H, $J = 11.5$ Hz, PhCH₂), 4.81 (d, 1H, $J = 11.5$ Hz, PhCH₂), 4.77 (d, 1H, $J = 11.5$ Hz, PhCH₂), 4.67 (d, 1H, $J = 11.5$ Hz, PhCH₂), 4.59 (d, 1H, $J = 11.5$ Hz, PhCH₂), 4.49 (d, 1H, $J = 11.5$ Hz, PhCH₂), 4.41 (dd, 1H, $J_{4,5} = 8.4$ Hz, H-4_{sph}), 4.39 (d, 1H, $J = 11.5$ Hz, PhCH₂), 4.17 (dd, 1H, $J_{2,3} = 9.8$ Hz, $J_{3,4} = 6.0$ Hz, H-3_{sph}), 4.11 (d, 1H, $J_{1a,1b} = 11.7$ Hz, $J_{1a,2} = 2.8$ Hz, H-1a_{sph}), 4.06 (m, 2H, H-2 and H-2_{sph}), 4.00 (dd, 1H, $J_{5,6a} = J_{5,6b} = 6.4$ Hz, H-5), 3.91-3.94 (m, 2H, H-3 and H-4), 3.63 (dd, 1H, $J_{1b,2} = 3.5$ Hz, H-1b_{sph}), 3.56 (dd, 1H, $J_{6a,6b} = 9.4$ Hz, H-6a), 3.36 (dd, 1H, H-6b), 1.98 (m, 2H, =CHCH₂), 1.80 (s, 3H, NHCOCH₃), 1.45 (s, 3H, C(CH₃)₂), 1.34 (s, 3H, C(CH₃)₂), 1.20-1.34 (m, 20H, alkane CH₂), 0.89 (t, 3H, $J = 6.8$ Hz, CH₃); ^{13}C NMR (125 MHz, CDCl_3) δ 169.0 (C=O), 138.7, 138.4, 138.4, 137.5, 136.0, 128.5, 128.4,

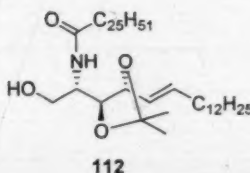
128.4, 128.3, 128.0, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 124.4, 108.3, 99.9, 79.0, 76.9, 75.8, 74.7, 74.6, 73.6, 73.4, 73.0, 70.8, 69.9, 69.6, 49.2, 32.4, 31.9, 29.7, 29.7, 29.6, 29.6, 29.5, 29.3, 29.1, 28.0, 25.7, 23.3, 22.7, 14.1; ESI HRMS m/z calcd for $C_{57}H_{77}NO_9Na$ ($M+Na^+$): 942.5491. Found: 942.5493.



(2*S*,3*S*,4*R*)-1-*O*-(α -D-galactopyranosyl)-2-acetamido-(5*E*)-octadecene-1,3,4-triol
(111)

To a solution of compound **109** (92 mg, 0.1 mmol) in a mixture of methanol (4 mL) and dichloromethane (2 mL) was added 20% Pd(OH)₂ on charcoal (15 mg). The mixture was stirred under an atmosphere of hydrogen at RT for 18 h. The mixture was filtered and then concentrated. The residue was redissolved in 80% aqueous acetic acid solution (7.5 mL) and heated at 80 °C for 1 h. The reaction was then quenched by amberlite IRA-400 basic resin. After filtration and concentration, the residue was purified by silica gel chromatography using 15% methanol in dichloromethane containing 1% NH₄OH to yield the desired compound **111** (47 mg, 91% over two steps): R_f = 0.12 (5:1 CH₂Cl₂-CH₃OH); $[\alpha]_D^{+82.6}$ (c 0.35, CH₃OH); ¹H NMR (500 MHz, CD₃OD) δ 4.86 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 4.24 (dd, 1H, J = 5.0 Hz, H-2_{sph}), 3.92 (dd, 1H, $J_{1a,1b}$ = 10.7 Hz, $J_{1a,2}$ = 4.6 Hz, H-1a_{sph}), 3.87 (d, 1H, J = 3.3 Hz, H-4), 3.82 (dd, 1H, $J_{5,6a}$ = $J_{5,6b}$ = 6.4 Hz, H-5), 3.77 (dd, 1H, $J_{2,3}$ = 10.1 Hz, H-2), 3.73 (dd, 1H, $J_{3,4}$ = 3.2 Hz, H-3), 3.67-3.73 (m, 2H, H-6a, H-6b), 3.65 (dd, 1H, $J_{1b,2}$ = 5.2 Hz, H-1b_{sph}), 3.55 (dd, 1H, J = 6.7 Hz, H-3_{sph}), 3.52 (ddd, 1H, J =

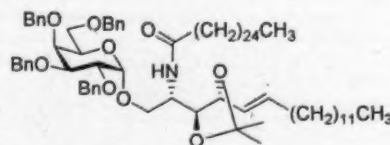
7.3 Hz, $J = 6.4$ Hz, $J = 1.8$ Hz, H-4_{sph}), 1.96 (s, 3H, NHCOCH₃), 1.67 (m, 1H, alkane CH₂), 1.54 (m, 1H, alkane CH₂), 1.24-1.40 (m, 24H, alkane CH₂), 0.89 (t, 3H, $J = 6.8$ Hz, CH₃); ¹³C NMR (125 MHz, CD₃OD) δ 172.9, 101.1, 75.8, 72.9, 72.7, 71.5, 71.1, 68.0, 62.8, 51.9, 33.6, 33.1, 30.8, 30.8, 30.5, 26.9, 23.7, 22.8, 14.4; ESI HRMS m/z Calcd for C₂₆H₅₁NO₉Na (M+Na⁺): 544.3456. Found 544.3455.



(2*S*,3*S*,4*R*)-2-(*N*-hexacosanoylamino)-3,4-*O*-isopropylidene-(5*E*)-octadecene-1,3,4-triol (112)

Compound **100** (30 mg, 0.075 mmol) was dissolved in a mixture of 0.5M Ba(OH)₂ solution and methanol (4 mL, 2:1 v/v). The mixture was stirred at 90 °C for 18 h. Then the mixture was extracted three times with ethyl acetate, and the combined extracts were concentrated for direct use in next step. To the above residue in dry THF (2 mL) were successively added hexacosanoic acid (32 mg, 0.08 mmol), EDCI (15 mg, 0.08 mmol) and HOBt (10.8 mg, 0.08 mmol). The mixture was stirred at 35-40 °C for 2 h (TLC showed that all starting material has gone). Concentration followed by chromatography of the residue on silica gel using 3:2 hexane/ethyl acetate as eluent provided the target compound **112** as a white solid (44.5 mg, 81%); $R_f = 0.24$ (3:1 toluene-ethyl acetate); $[\alpha]_D -1.25$ (c 0.76, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 5.93 (d, 1H, $J_{\text{NH,H-2}} = 8.2$ Hz, NH), 5.83 (dt, 1H, $J_{6,7} = 6.8$ Hz, H-6), 5.49 (ddd, 1H, $J_{5,6} = 15.3$ Hz, H-5), 4.66 (dd, 1H, $J_{4,5} = 7.4$ Hz, H-4), 4.25 (dd, 1H, $J_{3,4} = 6.7$ Hz, H-3), 4.30 (dddd, 1H, $J_{2,3} = 6.1$ Hz, H-2), 3.90

(dd, 1H, $J_{1a,1b} = 11.5$ Hz, $J_{1a,2} = 3.5$ Hz, H-1a), 3.68 (dd, 1H, $J_{1b,2} = 3.2$ Hz, H-1b), 2.67 (s, broad, 1H, OH), 2.17 (t, 2H, $J = 7.6$ Hz, C(O)CH₂), 2.06 (q, 2H, $J = 6.8$ Hz, =CHCH₂), 1.56-1.64 (m, 4H, alkane CH₂), 1.50 (s, 3H, C(CH₃)₂), 1.36 (s, 3H, C(CH₃)₂), 1.20-1.40 (m, 62H, alkane CH₂), 0.88 (t, 3H, $J = 6.8$ Hz, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 173.1, 137.1, 124.3, 108.5, 78.9, 78.8, 63.3, 50.7, 36.8, 32.4, 31.9, 29.7, 29.6, 29.6, 29.5, 29.5, 29.4, 29.3, 29.0, 27.3, 25.6, 24.8, 22.7, 14.1; ESI HRMS m/z calcd for C₄₇H₉₁NO₄Na (M+Na⁺): 756.6840. Found 756.6840.

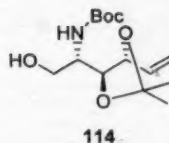


113

(2*S*,3*S*,4*R*)-2-(*N*-hexacosanoylamino)-3,4-*O*-isopropylidene-1-*O*-(2,3,4,6-tetra-*O*-benzyl- α -D-galactopyranosyl)-(5*E*)-octadecene-1,3,4-triol (113)

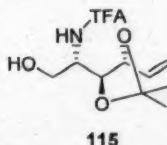
To a solution of 1-*O*-acetyl-2,3,4,6-tetra-*O*-benzyl-D-galactopyranoside **104** (203 mg, 0.35 mmol) in dry dichloromethane (3 mL) was added dropwise iodotrimethylsilane (70 mg, 0.35 mmol) at 0 °C. The mixture was stirred at 0 °C for 20 min. and the reaction was stopped by addition of dry toluene (3 mL). The mixture was co-evaporated three times with dry toluene to give a pale yellow residue, which was dissolved in dry benzene (1 mL) under argon. In a separate flask, tetrabutylammonium iodide (385 mg, 1.05 mmol), acceptor **112** (86 mg, 0.117 mmol), DIPEA (45 mg, 0.35 mmol) and 4Å molecular sieves were added into a benzene solution (2 mL). The mixture was stirred under argon at 65 °C for 10 min., followed by addition of freshly prepared galactosyl iodide in benzene. The mixture was stirred under argon at 65 °C for 2 h. The reaction was stopped by addition of

ethyl acetate (10 mL) and the mixture was cooled to 0 °C to precipitate tetrabutylammonium iodide. The white precipitate and molecular sieves were filtered off. After concentration, the residue was purified by chromatography on silica gel using hexane/ethyl acetate (5:1) as eluent to afford exclusively the desired α -anomer **113** as a white solid (103 mg, 70%): R_f = 0.61 (5:1 toluene-ethyl acetate); $[\alpha]_D^{+50.0}$ (c 1.38, CHCl_3); ^1H NMR (600 MHz, CDCl_3) δ 7.25-7.40 (m, 20H, ArH), 6.11 (d, 1H, $J_{\text{NH,H-2}}$ = 9.1 Hz, NH), 5.60 (dt, 1H, $J_{6,7}$ = 6.7 Hz, H-6_{sph}), 5.39 (dddd, 1H, $J_{5,6}$ = 15.2 Hz, J = 1.3 Hz, H-5_{sph}), 4.93 (d, 1H, J = 11.5 Hz, PhCH_2), 4.91 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 4.82 (d, 1H, J = 11.7 Hz, PhCH_2), 4.79 (d, 1H, J = 11.4 Hz, PhCH_2), 4.74 (d, 1H, J = 11.7 Hz, PhCH_2), 4.67 (d, 1H, J = 11.4 Hz, PhCH_2), 4.58 (d, 1H, J = 11.6 Hz, PhCH_2), 4.49 (d, 1H, J = 11.8 Hz, PhCH_2), 4.40 (dd, 1H, $J_{4,5}$ = 8.2 Hz, H-4_{sph}), 4.38 (d, 1H, J = 11.8 Hz, PhCH_2), 4.18 (dd, 1H, $J_{3,4}$ = 5.9 Hz, J = 9.4 Hz, H-3_{sph}), 4.03-4.08 (m, 3H, H-2, H-2_{sph} and H-1a_{sph}), 3.98 (dd, 1H, $J_{5,6a}$ = 6.3 Hz, $J_{5,6b}$ = 6.3 Hz, H-5), 3.90-3.94 (m, 2H, H-4 and H-3), 3.63 (dd, 1H, $J_{1a,1b}$ = 12.1 Hz, J = 3.1 Hz, H-1b_{sph}), 3.54 (dd, 1H, $J_{6a,6b}$ = 9.4 Hz, H-6a), 3.38 (dd, 1H, H-6b), 1.90-2.04 (m, 4H, alkane CH_2), 1.48-1.58 (m, 2H, alkane CH_2), 1.45 (s, 3H, $\text{C}(\text{CH}_3)_2$), 1.34 (s, 3H, $\text{C}(\text{CH}_3)_2$), 1.20-1.32 (m, 64H, alkane CH_2), 0.89 (t, 6H, J = 6.7 Hz, alkyl $2\times\text{CH}_3$); ^{13}C NMR (125 MHz, CDCl_3) δ 172.0, 138.7, 138.4, 137.6, 136.1, 128.5, 128.4, 128.4, 128.3, 128.2, 128.2, 128.1, 128.0, 127.9, 127.9, 127.9, 127.8, 127.8, 127.7, 127.5, 127.4, 124.4, 108.2, 99.6, 79.0, 78.9, 76.9, 75.9, 74.8, 74.7, 73.7, 73.4, 70.4, 69.8, 69.5, 49.0, 36.8, 32.5, 31.9, 29.7, 29.7, 29.7, 29.7, 29.6, 29.6, 29.5, 29.4, 29.4, 29.4, 29.1, 28.0, 25.7, 25.5, 22.7, 14.1; ESI HRMS m/z calcd for $\text{C}_{81}\text{H}_{125}\text{NO}_9\text{Na}$ ($\text{M}+\text{Na}^+$): 1278.9247. Found 1278.9249.



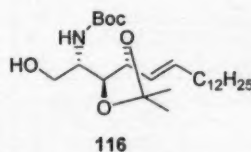
(2*S*,3*S*,4*R*)-2-*N*-(*tert*-butoxycarbonyl)amino-3,4-*O*-isopropylidene--hex-5-ene-1,3,4-triol (114)

Compound **98** (130 mg, 0.57 mmol) was dissolved in 0.5M Ba(OH)₂ solution (4 mL) and the mixture was heated at 90 °C for 18 h. The mixture was concentrated and filtered through a short silica pad. After concentration, the residue was dissolved in a mixture of ethanol and water (5 mL, 2:1, v/v). To the mixture was added 1M NaOH (0.85 mL, 0.85 mmol) and di-*tert*-butyldicarbonate (185 mg, 0.85 mmol). The mixture was stirred at RT for 2 h and the reaction was quenched by aq. NH₄Cl. Then the mixture was extracted three times with dichloromethane (10 mL), and this solution was washed with brine and dried over anhydrous Na₂SO₄. Concentration and chromatography of the residue (4:1 hexane/ethyl acetate) on silica gel afforded the desired product **114** as a white foam (136 mg, 94%): *R*_f = 0.28 (2:1 hexane-ethyl acetate); [α]_D -7.4 (*c* 1.1, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 5.90 (ddd, 1H, *J*_{5,6t} = 17.3 Hz, *J*_{5,6c} = 10.3 Hz, H-5), 5.40 (ddd, 1H, *J*_{6t,6c} = 1.4 Hz, H-6t), 5.28 (ddd, H-6c), 4.94 (s, broad, 1H, NH), 4.68 (dd, 1H, *J*_{4,5} = 6.9 Hz, H-4), 4.25 (d, *J*_{2,3} = *J*_{3,4} = 5.7 Hz, H-3), 3.84 (dd, 1H, *J*_{1a,1b} = 10.7 Hz, *J*_{1a,2} = 1.6 Hz, H-1a), 3.68-3.75 (dd, 2H, *J* = 2.5 Hz, H-2 and H-1b), 1.50 (s, 3H, C(CH₃)₂), 1.37 (s, 3H, C(CH₃)₂), 1.43 (s, 9H, C(CH₃)₃); ¹³C NMR (125 MHz, CDCl₃) δ 155.3, 133.0, 118.8, 108.9, 79.7, 78.8, 78.6, 63.4, 51.4, 28.3, 27.4, 25.0; ESI HRMS *m/z* calcd for C₁₄H₂₅NO₅Na (M+Na⁺): 310.1625. Found 310.1624. Anal calcd for C₁₄H₂₅NO₅: C 58.52, H 8.77, N 4.87. Found: C 58.72, H 8.83, N 4.77.



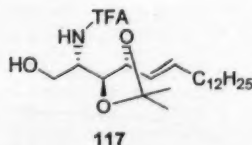
(2*S*,3*S*,4*R*)-3,4-*O*-isopropylidene-2-trifluoroacetamido-hex-5-ene-1,3,4-triol (115)

Compound **98** was dissolved in 0.5M Ba(OH)₂ solution (5 mL) and the mixture was heated at 90 °C for 18 h. The mixture was concentrated and filtered through a short silica pad. After the removal of solvent, the residue was dissolved in dry methanol and methyl trifluoroacetate (260 mg, 2 mmol) and Et₃N (0.14 mL, 1 mmol) were added. The mixture was stirred at RT for 3 h, then diluted with dichloromethane (10 mL) and the solution was washed with brine and dried over anhydrous Na₂SO₄. After concentration, the residue was purified by column chromatography (2:1 hexane/ethyl acetate) to afford the desired product **115** as a white foam (241 mg, 87%); *R*_f = 0.27 (3:2 hexane-ethyl acetate); [α]_D -36.5 (*c* 0.6, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 6.83 (s, broad, NH), 5.84 (ddd, 1H, *J*_{5,6t} = 17.1 Hz, *J*_{5,6c} = 10.4 Hz, H-5), 5.44 (d, 1H, H-6t), 5.33 (d, 1H, H-6c), 4.74 (dd, 1H, *J*_{4,5} = 6.9 Hz, H-4), 4.34 (dd, 1H, *J*_{3,4} = 6.4 Hz, H-3), 4.08 (ddd, 1H, *J*_{2,3} = 6.3 Hz, H-2), 4.00 (dd, 1H, *J*_{1a,1b} = 11.5 Hz, *J*_{1a,2} = 2.8 Hz, H-1a), 3.70 (dd, 1H, *J*_{1b,2} = 3.4 Hz, H-1b), 1.53 (s, 3H, C(CH₃)₂), 1.39 (s, 3H, C(CH₃)₂); ¹³C NMR (125 MHz, CDCl₃) δ 156.9, 56.6, 132.1, 119.6, 116.8, 114.5, 109.2, 78.4, 77.7, 61.9, 50.7, 27.3, 24.8; ESI HRMS *m/z* calcd for C₁₁H₁₆NO₄F₃Na (M+Na⁺): 306.0924. Found 306.0925. Anal calcd for C₁₁H₁₆NO₄F₃: C 46.64, H 5.69, N 4.95. Found: C 46.91, H 5.95, N 4.74.



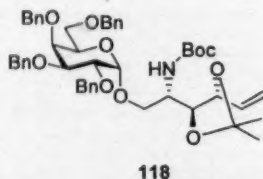
(2*S*,3*S*,4*R*)-2-*N*-(*tert*-butoxycarbonyl)amino-3,4-*O*-isopropylidene-(5*E*)-octadecene-1,3,4-triol (116)

To a solution of compound **114** (70 mg, 0.244 mmol) and 1-tetradecene (0.32 mL, 12.6 mmol) in dry dichloromethane (10 mL), Grubbs' second generation catalyst (20%, 41.4 mg) was added in four portions (protocol B) under reflux. The reaction was continued for 18 h under gentle reflux. After concentration, the residue was purified by chromatography on silica using hexane/ethyl acetate (4:1) as eluent affording the target product **116** as a white solid (85 mg, 77%, *E/Z* = ~12:1): R_f = 0.34 (2:1 hexane-ethyl acetate); $[\alpha]_D +2.1$ (c 0.88, CHCl_3); ^1H NMR (600 MHz, CDCl_3) δ 5.83 (dt, 1H, $J_{6,7}$ = 6.8 Hz, H-6), 5.50 (dd, 1H, $J_{5,6}$ = 15.3 Hz, H-5), 4.93 (s, broad, 1H, NH), 4.65 (dd, 1H, $J_{4,5}$ = 7.2 Hz, H-4), 4.19 (dd, 1H, $J_{2,3}$ = $J_{3,4}$ = 6.3 Hz, H-3), 3.86 (dd, 1H, $J_{1a,1b}$ = 11.0 Hz, $J_{1a,2}$ = 3.0 Hz, H1a), 3.70 (dd, 2H, J = 9.3 Hz, J = 3.2 Hz, H-2 and H1b), 2.06 (t, 2H, J = 7.0 Hz, =CHCH₂), 1.49 (s, 3H, -C(CH₃)₂), 1.36 (s, 3H, -C(CH₃)₂), 1.44 (s, 9H, -C(CH₃)₃), 1.20–1.40 (m, 20H, alkane CH₂), 0.88 (t, 3H, J = 6.9 Hz, CH₃); ^{13}C NMR (125 MHz, CDCl_3) δ 155.4, 136.9, 124.2, 108.5, 78.9, 78.7, 63.5, 51.6, 32.5, 31.9, 29.7, 29.6, 29.6, 29.6, 29.5, 29.5, 29.3, 29.3, 29.0, 28.4, 27.4, 25.0, 22.7, 14.1; ESI HRMS m/z calcd for $\text{C}_{26}\text{H}_{49}\text{NO}_5\text{Na}$ ($\text{M}+\text{Na}^+$): 478.3503. Found 478.3507.



(2*S*,3*S*,4*R*)-3,4-*O*-isopropylidene-2-trifluoroacetamido-(5*E*)-octadecene-1,3,4-triol (117)

To a solution of compound **115** (40 mg, 0.14 mmol) and 1-tetradecene (0.18 mL, 0.7 mmol) in dry dichloromethane (5 mL), Grubbs' second generation catalyst (20%, 23.7 mg) was added in four portions (protocol B) under reflux. The reaction was continued for 18 h under gentle reflux. Concentration followed by chromatography of the residue on silica gel (4:1 hexane/ethyl acetate) afforded the target product **117** as a white solid (53 mg, 83%): $R_f = 0.33$ (2:1 hexane-ethyl acetate); $[\alpha]_D -4.06$ (c 1.1, CHCl_3); ^1H NMR (500 MHz, CDCl_3) δ 6.79 (d, 1H, $J_{\text{NH,H-2}} = 8.2$ Hz, NH), 5.85 (dt, 1H, $J_{6,7} = 6.7$ Hz, H-6), 5.44 (dd, 1H, $J_{5,6} = 15.3$ Hz, H-5), 4.70 (dd, 1H, $J_{4,5} = 7.3$ Hz, H-4), 4.27 (dd, 1H, $J_{3,4} = 6.5$ Hz, H-3), 4.09 (ddd, 1H, $J_{2,3} = 6.0$ Hz, H-2), 4.00 (dd, $J_{1a,1b} = 11.5$ Hz, $J_{1a,2} = 2.7$ Hz, H-1a), 3.70 (dd, 1H, $J_{1b,2} = 3.4$ Hz, H-1b), 2.04 (q, 2H, $J = 7.1$ Hz, $=\text{CCH}_2$), 1.51 (s, 3H, $-\text{C}(\text{CH}_3)_2$), 1.38 (s, 3H, $-\text{C}(\text{CH}_3)_2$), 1.20-1.40 (m, 20H, alkane CH_2), 0.88 (t, 3H, $J = 6.8$ Hz, alkyl CH_3); ^{13}C NMR (125 MHz, CDCl_3) δ 137.8, 123.3, 108.9, 78.6, 77.7, 62.0, 50.8, 32.3, 31.9, 29.7, 29.6, 29.6, 29.4, 29.3, 29.3, 28.8, 27.3, 24.8, 22.7, 14.1; ESI HRMS m/z calcd for $\text{C}_{23}\text{H}_{40}\text{NO}_4\text{F}_3\text{Na}$ ($\text{M}+\text{Na}^+$): 474.2802. Found 474.2804. Anal calcd for $\text{C}_{23}\text{H}_{40}\text{NO}_4\text{F}_3$: C 61.18, H 8.93, N 3.10. Found: C 61.42, H 8.72, N 3.28.

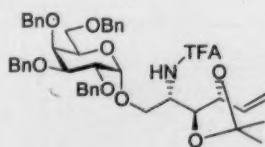


(2*S*,3*S*,4*R*)-2-*N*-(*tert*-butoxycarbonyl)amino-3,4-*O*-isopropylidene-1-*O*-(2,3,4,6-tetra-*O*-benzyl- α -D-galactopyranosyl)-hex-5-ene-1,3,4-triol (118)

To a solution of 1-*O*-acetyl-2,3,4,6-tetra-*O*-benzyl- α -D-galactopyranoside **104** (290 mg, 0.50 mmol) in dry dichloromethane (3 mL) was added dropwise iodotrimethylsilane (100

mg, 0.5 mmol) at 0 °C. The mixture was stirred at 0 °C for 20 min and the reaction was stopped by addition of dry toluene (3 mL). The mixture was co-evaporated three times with dry toluene to give a pale yellow residue, which was dissolved in dry benzene (1 mL) under argon. In a separate flask, tetrabutylammonium iodide (549 mg, 1.49 mmol), acceptor **114** (50 mg, 0.17 mmol), DIPEA (90 μ L, 1.5 mmol) and 4Å molecular sieves were added into a benzene solution (2 mL). The mixture was stirred at 65 °C for 10 min., followed by addition of freshly prepared galactosyl iodide in benzene. The mixture was stirred at 65 °C for 1-2 h. The reaction was stopped by addition of ethyl acetate (10 mL) and the mixture was cooled to 0 °C to precipitate tetrabutylammonium iodide. The white precipitate and molecular sieves were filtered off. Concentration followed by column chromatography of the residue using 15:1 to 10:1 toluene/ethyl acetate as eluent afforded the desired product **118** as white foam (109 mg, 79%, α/β = ~10:1): R_f = 0.58 (4:1 toluene-ethyl acetate); $[\alpha]_D +52.7$ (c 1.64, CHCl_3); ^1H NMR (500 MHz, CDCl_3) δ 7.22-7.42 (m, 20H, ArH), 5.84 (ddd, 1H, $J_{5,6t}$ = 17.3 Hz, $J_{5,6c}$ = 10.3 Hz, H-5_{sph}), 5.29 (d, 1H, H-6t_{sph}), 5.16 (d, 1H, $J_{6c,5}$ = 10.3 Hz, H-6c_{sph}), 5.10 (d, 1H, $J_{\text{NH,H-2}}$ = 9.6 Hz, NH), 4.94 (d, 1H, J = 11.5 Hz, PhCH₂), 4.93 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1), 4.84 (d, 1H, J = 11.5 Hz, PhCH₂), 4.79 (d, 1H, J = 11.5 Hz, PhCH₂), 4.75 (d, 1H, J = 11.5 Hz, PhCH₂), 4.68 (d, 1H, J = 11.5 Hz, PhCH₂), 4.58 (d, 1H, J = 11.5 Hz, PhCH₂), 4.50 (d, 1H, J = 11.5 Hz, PhCH₂), 4.34 (dd, 1H, $J_{4,5}$ = 6.6 Hz, H-4_{sph}), 4.38 (d, 1H, J = 11.5 Hz, PhCH₂), 4.24 (dd, 1H, $J_{3,4}$ = 6.2 Hz, H-3_{sph}), 4.05 (dd, 1H, $J_{2,3}$ = 9.9 Hz, H-2), 3.92-4.00 (m, 3H, H-3, H-4 and H-5), 3.90 (m, 1H, H-1a_{sph}), 3.74 (m, 1H, H-2_{sph}), 3.68 (dd, 1H, $J_{1b,1a}$ = 11.1 Hz, $J_{1b,2}$ = 2.6 Hz, H-1b_{sph}), 3.53 (dd, 1H, $J_{6a,6b}$ = 9.2 Hz, $J_{6a,5}$ = 6.3 Hz, H-6a), 3.47 (dd, 1H, $J_{6b,5}$ = 6.2 Hz, H-6b), 1.46 (s, 3H, C(CH₃)₂), 1.32 (s, 3H, C(CH₃)₂), 1.42 (s, 9H, C(CH₃)₃); ^{13}C

NMR (125 MHz, CDCl_3) δ 154.8, 138.8, 138.6, 138.6, 137.8, 133.1, 130.8, 129.0, 129.0, 128.5, 128.4, 128.3, 128.3, 128.3, 128.2, 128.2, 128.1, 128.0, 127.9, 127.7, 127.6, 127.5, 127.5, 127.4, 118.0, 108.6, 99.1, 79.0, 78.9, 76.8, 76.3, 74.9, 74.8, 74.7, 73.6, 73.1, 69.8, 69.6, 69.3, 68.8, 58.0, 50.5, 28.4, 27.9, 25.6; HRMS m/z calcd for $\text{C}_{48}\text{H}_{59}\text{NO}_{10}\text{Na}$ ($\text{M}+\text{Na}^+$): 832.4031. Found: 832.4035.

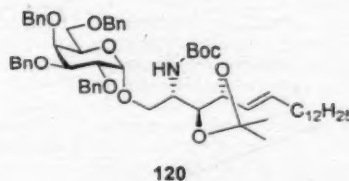


119

(2*S*,3*S*,4*R*)-3,4-*O*-isopropylidene-1-*O*-(2,3,4,6-tetra-*O*-benzyl- α -D-galactopyranosyl)-2-(trifluoroacetamido)-hex-5-ene-1,3,4-triol (119)

To a solution of 1-*O*-acetyl-2,3,4,6-tetra-*O*-benzyl-D-galactopyranoside **104** (580 mg, 1 mmol) in dry dichloromethane (6 mL) was added dropwise iodotrimethylsilane (200 mg, 1 mmol) at 0 °C. The mixture was stirred at 0 °C for 20 min and the reaction was stopped by addition of dry toluene (3 mL). The mixture was co-evaporated three times with dry toluene to give a pale yellow residue, which was dissolved in dry dichloromethane (2 mL) under argon. In a separate flask, tetrabutylammonium iodide (1.1 g, 3 mmol), acceptor **115** (90 mg, 0.31 mmol), DIPEA (170 μL , 1 mmol) and 4Å molecular sieves were added to dry dichloromethane (4 mL). The mixture was stirred under reflux until tetrabutylammonium iodide was dissolved. Then freshly prepared galactosyl iodide in dichloromethane was added and the mixture was stirred under gentle reflux for 2 h. The reaction was stopped by addition of ethyl acetate (10 mL) and the mixture was cooled to 0 °C to precipitate tetrabutylammonium iodide. After the removal of the white precipitate

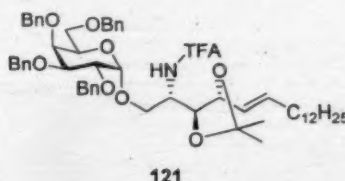
and molecular sieves by filtration and concentration of filtrate, the residue was subjected to column chromatography (5:1 hexane/ethyl acetate) affording exclusively the desired α -anomer **119** as white solid (221 mg, 88%): $R_f = 0.63$ (4:1 toluene-ethyl acetate); $[\alpha]_D^{+137}$ (c 0.74, CHCl_3); ^1H NMR (600 MHz, CDCl_3) δ 7.58 (d, 1H, $J_{\text{NH,H-2}} = 9.2$ Hz, NH), 7.24-7.41 (m, 20H, ArH), 5.60 (ddd, 1H, $J_{5,6t} = 17.1$ Hz, $J_{5,6c} = 10.4$ Hz, H-5_{sph}), 5.18 (ddd, 1H, $J_{6t,6c} = 1.3$ Hz, H-6t_{sph}), 5.13 (d, 1H, H-6c_{sph}), 4.93 (d, 1H, $J = 11.6$ Hz, PhCH₂), 4.85 (d, 1H, $J_{1,2} = 3.8$ Hz, H-1), 4.85 (d, 1H, $J = 11.6$ Hz, PhCH₂), 4.80 (d, 1H, $J = 11.5$ Hz, PhCH₂), 4.74 (d, 1H, $J = 11.5$ Hz, PhCH₂), 4.64 (d, 1H, $J = 11.6$ Hz, PhCH₂), 4.59 (d, 1H, $J = 11.6$ Hz, PhCH₂), 4.50 (d, 1H, $J = 11.5$ Hz, PhCH₂), 4.36 (d, 1H, $J = 11.8$ Hz, PhCH₂), 4.32 (dd, 1H, $J_{4,5} = 6.3$ Hz, H-4_{sph}), 4.28 (dd, 1H, $J_{3,4} = 6.1$ Hz, H-3_{sph}), 4.15 (dd, 1H, $J_{1a,1b} = 12.1$ Hz, $J_{1a,2} = 3.1$ Hz, H-1a_{sph}), 4.06 (dd, 1H, $J_{2,3} = 9.5$ Hz, H-2), 3.98 (dddd, 1H, $J_{2,3} = 9.5$ Hz, H-2_{sph}), 3.96 (dd, 1H, $J = 6.8$ Hz, 6.8 Hz, H-5), 3.90 (m, 1H, H-4), 3.88 (d, 1H, $J = 2.9$ Hz, H-3), 3.60 (dd, 1H, $J_{1b,2} = 2.8$ Hz, H-1b_{sph}), 3.53 (dd, 1H, $J_{6a,6b} = 9.5$ Hz, $J_{6a,5} = 7.3$ Hz, H-6a), 3.34 (dd, 1H, $J_{6b,5} = 4.9$ Hz, H-6b), 1.46 (s, 3H, $\text{C}(\text{CH}_3)_2$), 1.34 (s, 3H, $\text{C}(\text{CH}_3)_2$); ^{13}C NMR (125 MHz, CDCl_3) δ 156.3 (q, $J_{\text{C,F}} = 36.6$ Hz), 138.5, 138.3, 138.2, 137.4, 132.0, 128.5, 128.4, 128.4, 128.0, 128.0, 128.0, 127.8, 127.7, 127.6, 118.8, 115.8 (q, $J_{\text{C,F}} = 288$ Hz), 99.9, 78.8, 78.4, 76.8, 75.1, 74.6, 74.6, 73.9, 73.6, 73.0, 70.2, 69.7, 69.6, 50.0, 27.8, 25.5; ESI HRMS m/z calcd for $\text{C}_{45}\text{H}_{50}\text{NO}_9\text{F}_3\text{Na}$ ($\text{M} + \text{Na}^+$): 828.3330. Found: 828.3332. Anal. Calcd for $\text{C}_{45}\text{H}_{50}\text{NO}_9\text{F}_3$: C 67.07, H 6.25, N 1.74. Found: C 67.04, H 6.47, N 2.00.



(2*S*,3*S*,4*R*)-2-*N*-(*tert*-butoxycarbonyl)amino-3,4-*O*-isopropylidene-1-*O*-(2,3,4,6-tetra-*O*-benzyl- α -D-galactopyranosyl)-(5*E*)-octadecene-1,3,4-triol (120)

To a solution of compound **118** (120 mg, 0.148 mmol) and 1-tetradecene (0.19 mL, 0.74 mmol) in dry dichloromethane (6 mL) was added Grubbs' second generation catalyst (20%, 23.2 mg) in four portions (protocol B) under reflux. The reaction was continued for 18 h under gentle reflux. Concentration followed by column chromatography of the residue using 20:1 to 10:1 hexane/ethyl acetate as eluent yielded the desired product **120** as a white solid (110 mg, 76%); $R_f = 0.53$ (3:1 hexane-ethyl acetate); $[\alpha]_D +44.9$ (c 1.33, CHCl₃); ¹H NMR (500 MHz, CD₂Cl₂) δ 7.25-7.42 (m, 20H, ArH), 5.68 (dt, 1H, $J_{6,7} = 6.8$ Hz, H-6_{sph}), 5.45 (ddd, $J_{5,6} = 15.2$ Hz, $J_{5,4} = 8.0$ Hz, $J = 1.4$ Hz, H-5_{sph}), 4.99 (d, 1H, $J_{NH,H-2} = 9.7$ Hz, NH), 4.92 (d, 1H, $J_{1,2} = 3.3$ Hz, H-1), 4.91 (d, 1H, $J = 11.5$ Hz, PhCH₂), 4.82 (d, 1H, $J = 11.5$ Hz, PhCH₂), 4.77 (d, 1H, $J = 11.5$ Hz, PhCH₂), 4.75 (d, 1H, $J = 11.5$ Hz, PhCH₂), 4.67 (d, 1H, $J = 11.5$ Hz, PhCH₂), 4.52 (d, 2H, $J = 11.5$ Hz, PhCH₂), 4.43 (m, 2H, PhCH₂ and H-4_{sph}), 4.12 (d, 1H, $J_{3,2} = 9.4$ Hz, $J_{3,4} = 6.0$ Hz, H-3_{sph}), 3.96-4.02 (m, H-2, H-5 and H-3/H-4), 3.92 (dd, 1H, $J = 10.3$ Hz, $J = 3.0$ Hz, H3/H4), 3.80 (dd, 1H, $J_{1a,1b} = 10.3$ Hz, $J_{1a,2} = 2.2$ Hz, H-1a_{sph}), 3.72 (broad d, 1H, $J = 8.7$ Hz, H-2_{sph}), 3.68 (dd, 1H, $J_{1b,2} = 2.7$ Hz, H-1b), 3.51-3.57 (m, 2H, H-6a_{sph} and H-6b_{sph}), 2.00 (t, 2H, $J = 7.7$ Hz, =CHCH₂), 1.45 (s, 3H, C(CH₃)₂), 1.30 (s, 3H, C(CH₃)₂), 1.42 (s, 9H, C(CH₃)₃), 1.25-1.40 (m, 20H, alkane CH₂), 0.88 (t, 3H, $J = 6.8$ Hz, alkyl CH₃); ¹³C NMR (125 MHz, CD₂Cl₂) δ 155.0, 139.4, 139.3, 139.2, 128.7, 128.6, 128.6, 128.5, 128.5, 128.2, 128.2,

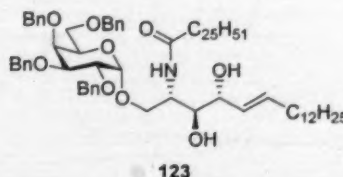
128.1, 128.0, 127.9, 127.8, 127.8, 125.1, 120.5, 99.2, 79.3, 79.3, 77.3, 76.7, 76.6, 75.8, 75.3, 73.8, 73.3, 73.3, 73.2, 69.9, 69.6, 51.0, 32.9, 32.3, 30.1, 30.0, 30.0, 29.9, 29.8, 29.7, 29.6, 28.6, 28.5, 28.1, 25.8, 23.1, 14.3; ESI HRMS m/z calcd for $C_{60}H_{83}NO_{10}Na$ ($M+Na^+$): 1000.5909. Found: 1000.5910.



(2*S*,3*S*,4*R*)-3,4-*O*-isopropylidene-1-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-2-(trifluoroacetamido)-(5*E*)-octadecene-1,3,4-triol (121)

To a solution of compound **119** (80 mg, 0.1 mmol) and 1-tetradecene (0.13 mL, 0.5 mmol) in dry dichloromethane (5 mL), Grubbs' second generation catalyst (20%, 17 mg) was added in four portions (protocol B) under reflux. The reaction was continued for 18 h under gentle reflux. After concentration, the residue was purified by chromatography on silica using hexane/ethyl acetate (8:1) as eluent to afford the target product **121** as a white solid (85 mg, 87%): R_f = 0.55 (3:1 hexane-ethyl acetate); $[\alpha]_D +9.6$ (c 0.68, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.46 (d, 1H, $J_{NH,H-2}$ = 9.5 Hz, NH), 7.24-7.42 (m, 20H, ArH), 5.80 (dt, 1H, $J_{6,7}$ = 6.7 Hz, H-6_{sph}), 5.24 (dddd, $J_{5,6}$ = 15.2 Hz, J = 1.4 Hz, H-5_{sph}), 4.91 (d, 1H, J = 11.5 Hz, PhCH₂), 4.85 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1), 4.84 (d, 1H, J = 11.5 Hz, PhCH₂), 4.80 (d, 1H, J = 11.5 Hz, PhCH₂), 4.74 (d, 1H, J = 11.4 Hz, PhCH₂), 4.62 (d, 1H, J = 11.5 Hz, PhCH₂), 4.58 (d, 1H, J = 11.6 Hz, PhCH₂), 4.50 (d, 1H, J = 11.5 Hz, PhCH₂), 4.34 (d, 1H, J = 12.0 Hz, PhCH₂), 4.30 (dd, 1H, $J_{4,5}$ = 8.0 Hz, H-4_{sph}), 4.20 (dd, 1H, $J_{3,4}$ = 6.0 Hz, H-3_{sph}), 4.14 (dd, 1H, $J_{1a,1b}$ = 12.0 Hz, $J_{1a,2}$ = 2.6 Hz, H-1a_{sph}), 4.06 (dd,

1H, $J_{2,3} = 9.3$ Hz, H-2), 4.01 (dddd, 1H, $J_{2,3} = 9.5$ Hz, H-2_{sph}), 3.95 (dd, 1H, $J_{5,6a} = 7.0$ Hz, $J_{5,6b} = 5.4$ Hz, H-5), 3.90 (m, 1H, H-4), 3.88 (d, 1H, $J_{3,4} = 2.8$ Hz, H-3), 3.59 (dd, 1H, $J_{1b,2} = 2.8$ Hz, H-1b_{sph}), 3.52 (dd, 1H, $J_{6a,6b} = 9.5$ Hz, H-6a), 3.34 (dd, 1H, H-6b), 1.92 (m, 2H, =CHCH₂), 1.44 (s, 3H, C(CH₃)₂), 1.34 (s, 3H, C(CH₃)₂), 1.20-1.40 (m, 20H, alkane CH₂), 0.88 (t, 3H, $J = 6.8$ Hz, alkyl CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 156.1 (q, $J_{C,F} = 37$ Hz), 138.5, 138.3, 138.2, 137.5, 136.9, 128.5, 128.4, 128.3, 128.3, 128.0, 127.95, 127.7, 127.6, 127.6, 123.2, 115.8 (q, $J_{C,F} = 288.5$ Hz), 108.5, 99.9, 78.8, 78.6, 76.8, 74.9, 73.9, 73.5, 73.0, 70.2, 69.7, 69.6, 49.9, 32.2, 31.9, 29.7, 29.6, 29.6, 29.5, 29.3, 29.3, 28.7, 27.9, 25.6, 22.7, 14.1; ESI HRMS m/z Calcd for C₅₇H₇₄NO₉F₃Na (M+Na⁺): 996.5208. Found: 996.5214.

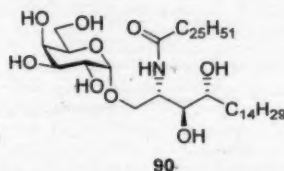


123

(2*S*,3*S*,4*R*)-3,4-*O*-isopropylidene-1-*O*-(2,3,4,6-tetra-*O*-benzyl- α -D-galactopyranosyl)-2-(*N*-hexacosanoylamino)-(5*E*)-octadecene-1,3,4-triol (123)

4 M HCl in dioxane (100 μ L) was added to a solution of compound **121** (60 mg, 0.062 mmol) in methanol (5 mL) and dichloromethane (1 mL). The mixture was stirred at RT for 18 h and quenched by amberlite IRA-400 basic resin. After filtration and concentration, the residue was redissolved in methanol (5 mL) and 1M NaOH solution (0.5 mL) was added. The mixture was stirred at 35 $^{\circ}$ C for 3-4 h (TLC showed that the reaction was complete) and the reaction was quenched by addition of amberlite IR-120 acidic resin. Filtration and concentration of the filtrate gave a pale-yellow residue, which

was dissolved in dry dichloromethane (2 mL). To this mixture was added hexacosanoic acid (40 mg, 0.091 mmol), EDCI (17 mg, 0.091 mmol) and HOBT (12 mg, 0.091 mmol). The mixture was stirred at 40 °C under argon for 5-6 h. Concentration and chromatography of the residue on silica gel using hexane/ethyl acetate (2:1) as eluent provided the desired compound **123** as a white solid (49 mg, 66% over three steps): R_f = 0.23 (2:1 hexane-ethyl acetate); $[\alpha]_D^{25} +26.8$ (c 0.93, CHCl_3); ^1H NMR (500 MHz, CDCl_3) δ 7.2-7.4 (m, 20H, ArH), 6.31 (d, 1H, $J_{\text{NH,H-2}} = 8.4$ Hz, NH), 5.72 (ddd, 1H, $J_{6,7a} = J_{6,7b} = 6.7$ Hz, H-6_{sph}), 5.49 (dd, 1H, $J_{5,6} = 15.4$ Hz, H-5_{sph}), 4.93 (d, 1H, $J = 11.4$ Hz, PhCH₂), 4.88 (d, 1H, $J_{1,2} = 3.1$ Hz, H-1), 4.87 (d, 1H, $J = 12.2$ Hz, PhCH₂), 4.79 (d, 1H, $J = 11.8$ Hz, PhCH₂), 4.75 (d, 1H, $J = 11.6$ Hz, PhCH₂), 4.71 (d, 1H, $J = 11.6$ Hz, PhCH₂), 4.57 (d, 1H, $J = 11.3$ Hz, PhCH₂), 4.48 (d, 1H, $J = 11.6$ Hz, PhCH₂), 4.39 (d, 1H, $J = 11.7$ Hz, PhCH₂), 4.18 (ddd, 1H, $J = 4.2$ Hz, H-2_{sph}), 4.04-4.08 (m, 2H, H-4_{sph} and H-2), 3.95 (d, 1H, $J = 2.4$ Hz, H-4), 3.84-3.92 (m, 4H, H-5, H-3, H-6a, H-1a_{sph}), 3.50-3.58 (m, 3H, H-3_{sph}, H-1b_{sph}, H-6b), 2.10 (t, 2H, $J = 7.4$ Hz, alkane CH₂), 2.03 (q, 2H, $J = 7.2$ Hz, alkane CH₂), 1.54-1.60 (m, 2H, alkane CH₂), 1.20-1.40 (m, 64H, alkane CH₂), 0.89 (t, 6H, $J = 6.8$ Hz, alkyl 2xCH₃); ^{13}C NMR (125 MHz, CD_3OD) δ 173.0, 138.4, 138.4, 137.8, 137.6, 134.2, 128.5, 128.4, 128.4, 128.4, 128.3, 128.2, 128.2, 128.2, 128.0, 127.9, 127.9, 127.8, 127.6, 127.6, 127.5, 99.3, 79.1, 76.0, 75.5, 74.7, 74.6, 74.1, 73.8, 73.6, 73.5, 72.8, 70.0, 69.7, 69.0, 49.7, 36.7, 32.4, 31.9, 29.7, 29.6, 29.6, 29.5, 29.5, 29.4, 29.4, 29.3, 29.2, 25.7, 22.7, 14.1; ESI HRMS m/z calcd for $\text{C}_{78}\text{H}_{121}\text{NO}_9\text{Na}$ ($\text{M}+\text{Na}^+$): 1238.8933. Found: 1238.8931.



(2*S*,3*S*,4*R*)-1-*O*-(α -D-Galactopyranosyl)-2-(*N*-hexacosanoylamino)-octadecane-1,3,4-triol (a-Galcer,KRN7000) (90)

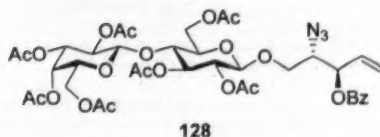
Method A: To a solution of **113** (51 mg, 0.04 mmol) in a mixture of methanol (2.5 mL) and dichloromethane (0.5 mL) was added 4M HCl in dioxane (50 μ L) at 0 °C. The mixture was stirred at RT for 18 h and the reaction was quenched by amberlite IRA-400 basic resin. After filtration and concentration, the residue was redissolved in a mixture of THF (2.5 mL)/methanol (0.5 mL)/acetic acid (0.15 mL), followed by addition of 20% Pd(OH)₂ on carbon (15 mg). The mixture was stirred under a hydrogen atmosphere for 18 h. Filtration and concentration of the filtrate gave a crude residue, which was purified by silica gel chromatography using 5% methanol in dichloromethane as eluent to afford the final product **90** as a white solid (25 mg, 72%).

Method B: Compound **120** (44 mg, 0.045 mmol) was dissolved in methanol (2 mL) and 20% Pd(OH)₂ (20 mg) on carbon was added. The mixture was stirred at RT for 18 h under an atmosphere of hydrogen. Filtration and concentration of filtrate gave a residue **122**, which was redissolved in aqueous 90% trifluoroacetic acid (3 mL). The mixture was stirred at RT for 18 h. After concentration, the residue was dissolved in dry methanol (2 mL) and hexacosanoic anhydride (40 mg, 0.05mmol) was added. The mixture was stirred at RT for 2 h. After concentration, the residue was purified by silica gel chromatography using 5% methanol in dichloromethane as eluent to provide the final product **90** as a white solid (22.5 mg, 58 % from **120**).

Method C: To a solution of **123** (30 mg, 0.025 mmol) in a mixture of THF (2.5 mL)/methanol (0.5 mL)/acetic acid (0.15 mL) was added 20% Pd(OH)₂ on carbon (15 mg). The mixture was stirred at RT under a hydrogen atmosphere for 18 h. After filtration and concentration of the filtrate, the residue was purified by chromatography on silica gel using 5% methanol in dichloromethane as eluent to afford the final product **90** as a white solid (20 mg, 96%).

R_f = 0.37 (5:1 CH₂Cl₂-CH₃OH); $[\alpha]_D$ +42.3 (c 0.35, pyridine); {lit.^[164] $[\alpha]_D$ +43.6 (c 1.0, pyridine)}; ¹H NMR (600 MHz, CDCl₃/CD₃OD = 4:1) δ 4.84 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1), 4.13 (ddd, 1H, $J_{2,3}$ = $J_{2,1a}$ = $J_{2,1b}$ = 4.5 Hz, H-2_{sph}), 3.88 (d, 1H, J = 3.3 Hz), 3.82 (dd, 1H, $J_{1a,1b}$ = 10.8 Hz, $J_{1a,2}$ = 4.7 Hz, H-1a_{sph}), 3.70-3.75 (m, 3H, H-2), 3.65-3.70 (m, 2H), 3.63 (dd, 1H, J = 6.0 Hz, J = 4.8 Hz), 3.61 (dd, 1H, $J_{1b,2}$ = 4.6 Hz, H-1b_{sph}), 3.45-3.50 (m, 2H, H-4_{sph} and H-3_{sph}), 2.34 (t, 2H, J = 7.4 Hz, C(O)CH₂), 1.40-1.64 (m, 4H, alkane CH₂), 1.16-1.34 (m, 68H, alkane CH₂), 0.82 (t, 6H, J = 7.0 Hz, alkyl 2xCH₃); ¹³C NMR (125 MHz, CDCl₃/CD₃OD = 4:1) δ 178.4, 103.6, 78.7, 75.9, 74.6, 74.3, 73.6, 72.8, 71.3, 65.8, 54.3, 40.4, 36.5, 33.5, 33.5, 33.5, 33.4, 33.3, 33.2, 33.2, 29.7, 29.7, 26.5, 17.8; ESI HRMS m/z calcd for C₅₀H₉₉NO₉Na (M+Na⁺): 880.7212. Found: 880.7217.

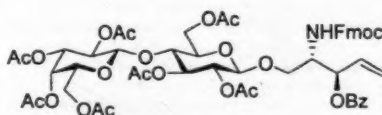
Chapter Five Part-II
Synthesis of β -Lactosylceramide



***O*-(2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-*O*-(2,3,6-tri-*O*-acetyl- β -D-glucopyranosyl)-(1 $\rightarrow$ 1)-(2*S*, 3*R*)-2-azido-3-*O*-benzoyl-pent-4-ene-1,3-diol (**128**)**

To a solution of lactosyl imidate (9.3 g, 11.9 mmol) and acceptor **126** (2.66 mg, 10.7 mmol) in dry dichloromethane (50 mL) containing 4Å molecular sieves (2 g), TMSOTf (13 μ L, 0.11 mmol) was added at RT. The mixture was then stirred at RT for 1 hour. After filtration and concentration, the residue was purified by silica gel chromatography (2:1 toluene/ethyl acetate) to provide the desired product **128** as a white solid (8.3 g, 90%); R_f = 0.17 (3:2 toluene-ethyl acetate); $[\alpha]_D$ -17.1 (c 0.42, CHCl_3); ^1H NMR (600 MHz, CDCl_3) δ 8.06 (dd, 2H, J = 8.2 Hz, J = 1.2 Hz, ArH), 7.58 (t, 1H, J = 7.5 Hz, ArH), 7.47 (t, 2H, J = 8.0 Hz, ArH), 5.94 (ddd, 1H, $J_{4,5t}$ = 17.4 Hz, $J_{4,5c}$ = 10.5 Hz, H-4_{sph}), 5.66 (dd, 1H, $J_{3,4}$ = 7.0 Hz, H-3_{sph}), 5.46 (ddd, 1H, $J_{5t,5c}$ = 1.2 Hz, H-5_{sph}), 5.41 (ddd, 1H, H-5_{sph}), 5.35 (dd, 1H, $J_{4',5'}$ = 1.1 Hz, H-4'), 5.20 (dd, 1H, $J_{3,4}$ = 9.1 Hz, H-3), 5.11 (dd, 1H, $J_{2',3'}$ = 10.4 Hz, H-2'), 4.96 (dd, 1H, $J_{3',4'}$ = 3.5 Hz, H-3'), 4.94 (dd, 1H, $J_{2,3}$ = 9.2 Hz, H-2), 4.54 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 4.49 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1'), 4.48 (dd, 1H, H-6a), 4.13 (dd, 1H, $J_{6a',6b'}$ = 11.0 Hz, H-6a'), 4.10 (dd, 1H, H-6b'), 4.06 (dd, 1H, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6b}$ = 5.2 Hz, H-6b), 3.97 (ddd, 1H, $J_{2,1b}$ = 8.3 Hz, $J_{2,3}$ = 4.2 Hz, H-2_{sph}), 3.92 (dd, 1H, $J_{1a,1b}$ = 10.3 Hz, $J_{1a,2}$ = 6.6 Hz, H-1a_{sph}), 3.87 (ddd,

^1H , $J_{5,6a} = J_{5,6b} = 7.3$ Hz, H-5'), 3.83 (dd, 1H, $J_{4,5} = 9.1$ Hz, H-4), 3.60-3.64 (m, 2H, H-5 and H-1b_{sph}), 2.15 (s, 3H, COCH₃), 2.08 (s, 3H, COCH₃), 2.06 (s, 3H, COCH₃), 2.05 (s, 6H, COCH₃), 2.04 (s, 3H, COCH₃), 1.96 (s, 3H, COCH₃); ^{13}C NMR (125MHz, CDCl₃) δ 170.3, 170.2, 170.1, 170.0, 169.7, 169.5, 169.0, 165.0, 133.3, 131.3, 129.8, 128.5, 120.8, 101.1, 100.3, 76.1, 74.5, 72.8, 72.8, 71.5, 71.0, 70.7, 69.1, 68.0, 66.6, 63.2, 61.8, 60.8, 20.8, 20.7, 20.7, 20.6, 20.6, 20.5; ESI HRMS m/z Calcd for C₃₈H₄₇N₃O₂₀Na: 888.2645. Found: 888.2642. Anal calcd for C₃₈H₄₇N₃O₂₀: C 52.72, H 5.47, N 4.85. Found: C 52.16, H 5.61, N 4.80.

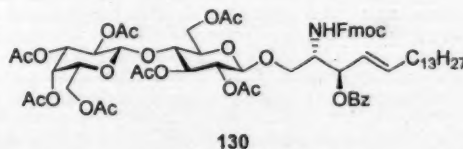


129

***O*-(2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-*O*-(2,3,6-tri-*O*-acetyl- β -D-glucopyranosyl)-(1 $\rightarrow$ 1)-(2*S*,3*R*)-3-*O*-benzoyl-2-(9-fluorenyl-methoxy-carbonyl)-pent-4-ene-1,3-diol (129)**

To a solution of **128** (700 mg, 0.809 mmol) in a mixture of pyridine (19 mL) and water (2 mL) was added triphenylphosphine (424 mg, 1.62 mmol). The mixture was stirred at RT for 1-2 h and FmocOSuccinimide (784 mg, 2.32 mmol) was then added. The mixture was stirred at RT until all starting material had been consumed. After concentration, the residue was dissolved in dichloromethane and washed with water, brine, dried over anhydrous Na₂SO₄. Concentration and silica gel chromatography (toluene/ethyl acetate 3:1) afforded the desired product **129** as a white solid (779 mg, 91%); $R_f = 0.35$ (3:2 toluene-ethyl acetate); $[\alpha]_D -15.4$ (c 0.42, CH₃OH); ^1H NMR (500 MHz, CDCl₃) δ 8.2 (d,

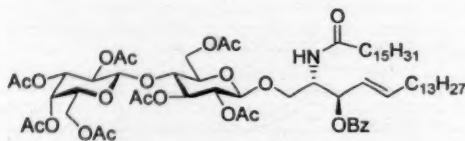
2H, $J = 8.4$ Hz, ArH), 7.76 (dd, 2H, $J = 7.5$ Hz, 2.6 Hz, ArH), 7.53-7.60 (m, 3H, ArH), 7.38-7.44 (m, 4H, ArH), 7.3 (d, 2H, $J = 7.2$ Hz, ArH), 5.90 (m, 1H, H-4_{sph}), 5.64 (dd, 1H, $J = 6.2$ Hz, H-3_{sph}), 5.42 (d, 1H, $J_{4,5t} = 17.2$ Hz, H-5_{t,sph}), 5.34 (dd, 2H, $J = 3.5, 1.0$ Hz, H-4'), 5.32 (d, 1H, $J = 10.6$ Hz, H-5_{sph}), 5.2 (dd, 1H, $J_{3,4} = 9.3$ Hz, H-3), 5.10 (dd, 1H, $J = 10.3$ Hz, 7.9 Hz, H-2'), 4.96 (dd, 1H, $J_{3,4} = 3.5$ Hz, H-3'), 4.92 (dd, 1H, $J_{2,3} = 9.3$ Hz, H-2), 4.60 (d, 2H, $J = 7.9$ Hz, H-1 and H-1'), 4.40 (dd, 1H, $J_{6a,6b} = 11.2$ Hz, H-6a), 4.22 (m, 1H, H-2_{sph}), 4.13 (dd, 1H, $J_{6a',6b'} = 11.3$ Hz, H-6a'), 4.03 (dd, 1H, $J_{1a,1b} = 10.4$ Hz, $J_{1b,2} = 4.3$ Hz, H-1_{sph}), 3.98 (dd, 1H, H-6b), 3.87 (ddd, 1H, $J_{5',6a'} = 6.4$ Hz, $J_{5',6b'} = 7.1$ Hz, H-5'), 3.78 (dd, 1H, $J_{4,5} = 9.6$ Hz, H-4), 3.68 (dd, 1H, $J_{1a,2} = 4.7$ Hz, H-1_{sph}), 3.58 (m, 1H, H-5), 2.15 (s, 3H, COCH₃), 2.07 (s, 3H, COCH₃), 2.05 (s, 3H, COCH₃), 2.03 (s, 3H, COCH₃), 1.98 (s, 9H, COCH₃); ¹³C NMR (125 MHz, CDCl₃) δ 170.3, 170.3, 170.1, 170.0, 169.7, 169.6, 169.1, 165.5, 155.9, 143.8, 143.8, 141.3, 141.3, 133.2, 132.6, 129.9, 129.7, 128.5, 128.4, 127.7, 127.0, 125.0, 120.0, 119.3, 101.1, 100.6, 76.1, 74.0, 72.7, 72.6, 71.6, 71.0, 70.7, 69.1, 67.9, 66.8, 66.7, 65.9, 61.9, 60.8, 52.9, 47.2, 20.8, 20.6, 20.6, 20.6, 20.5; ESI HRMS m/z Calcd for C₅₃H₅₉NO₂₂Na: 1084.3421. Found: 1084.3422.



***O*-(2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-*O*-(2,3,6-tri-*O*-acetyl- β -D-glucopyranosyl)-(1 $\rightarrow$ 1)-(2*S*,3*R*)-3-*O*-benzoyl-2-(9-fluorenyl-methoxy-carbonyl)-(4*E*)-octadecene-1,3-diol (130)**

To a solution of compound **129** (80 mg, 0.075 mmol) and 1-pentadecene (100 μ L, 0.37 mmol) in dry dichloromethane (4 mL), Grubbs' second generation catalyst (20%, 12.7 mg) was added in four portions (protocol B) under reflux. The reaction was continued for 18 h under gentle reflux. After concentration, the residue was purified by column chromatography (1:1 hexane and ethyl acetate) to provide the target compound **130** as a white solid (87 mg, 74%): R_f = 0.33 (3:2 toluene-ethyl acetate); $[\alpha]_D^{25}$ +3.6 (c 0.28, CHCl_3); ^1H NMR (500 MHz, CDCl_3) δ 8.01 (d, 2H, J = 8.4 Hz, ArH), 7.77 (dd, 2H, J = 7.5 Hz, J = 2.6 Hz, ArH), 7.57 (m, 3H, ArH), 7.41 (m, 4H, ArH), 7.30 (m, 2H, ArH), 5.90 (ddd, 1H, $J_{5,6t}$ = 6.5 Hz, $J_{5,6c}$ = 6.5 Hz, H-5_{sph}), 5.58 (dd, 1H, $J_{3,4}$ = 6.8 Hz, H-3_{sph}), 5.49 (dd, 1H, $J_{4,5}$ = 15.4 Hz, H-4_{sph}), 5.35 (d, 1H, J = 3.5 Hz, H-4'), 5.19 (dd, 1H, $J_{3,4}$ = 9.2 Hz, H-3), 5.10 (dd, 2H, $J_{2',3'}$ = 10.2 Hz, $J_{1',2'}$ = 8.4 Hz, NH, H-2'), 4.96 (dd, 1H, $J_{3',2'}$ = 10.3 Hz, $J_{3',4'}$ = 3.4 Hz, H-3'), 4.92 (dd, 1H, $J_{2,3}$ = 8.7 Hz, H-2), 4.42-4.52 (m, 3H, H-1, H-1' and H-1a_{sph}), 4.39 (d, 1H, $J_{6a,6b}$ = 11.9 Hz, H-6a), 4.33 (dd, 1H, $J_{1b,1a}$ = 10.2 Hz, $J_{1b,2}$ = 7.3 Hz, H-1b_{sph}), 4.16-4.24 (m, 2H, H-2_{sph} and H_{Fmoc}), 4.12 (dd, 1H, $J_{6a',6b'}$ = 11.2 Hz, H-6a'), 4.09 (dd, 1H, H-6b'), 4.12 (dd, 1H, J = 10.1, 4.1 Hz, H_{Fmoc}), 3.98 (dd, 1H, $J_{6b,6a}$ = 12.0 Hz, $J_{6b,5}$ = 5.3 Hz, H-6b), 3.86 (dd, 1H, $J_{5',6a'}$ = $J_{6b',5'}$ = 7.3 Hz, $J_{5',4'}$ = 0.8 Hz, H-5'), 3.78 (dd, 1H, $J_{4,3}$ = $J_{4,5}$ = 9.6 Hz, H-4), 3.67 (dd, 1H, J = 10.1, 4.4 Hz, H_{Fmoc}), 3.58 (m, 1H, H-5), 2.15 (s, 3H, COCH_3), 2.07 (s, 1H, COCH_3), 2.05 (s, 3H, COCH_3), 2.03 (s, 6H, COCH_3), 2.01 (m, 2H, alkane CH_2), 1.98 (s, 3H, COCH_3), 1.96 (s, 3H, COCH_3), 1.10-1.40 (m, 22H, alkane CH_2), 0.88 (t, 3H, J = 6.8 Hz, alkyl CH_3); ^{13}C NMR (125 MHz, CDCl_3) δ 170.3, 170.2, 170.1, 170.0, 169.7, 169.6, 169.0, 155.9, 143.9, 143.8, 141.3, 141.3, 137.5, 133.0, 130.2, 129.6, 128.4, 127.7, 127.0, 124.2, 120.0, 101.1, 100.6, 76.2, 74.1, 72.7, 71.7, 71.0, 70.7, 69.1, 67.9, 66.9, 66.6, 61.9, 60.8, 53.1, 47.2, 32.3, 31.9, 29.7,

29.6, 29.4, 29.3, 29.2, 28.9, 22.7, 20.8, 20.6, 20.6, 20.5, 14.1; ESI HRMS m/z Calcd for $C_{66}H_{85}NO_{22}Na$: 1266.5455. Found: 1266.5453.

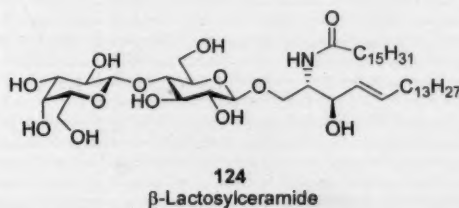


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***O*-(2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-*O*-(2,3,6-tri-*O*-acetyl- β -D-glucopyranosyl)-(1 $\rightarrow$ 1)-(2*S*, 3*R*)-3-benzoyl-2-octadecanamido-(4*E*)-octadecene-1,3-diol (131)**

Compound **130** (70 mg, 0.056 mmol) was treated with a mixture of 20% piperidine in dry DMF (5 mL) for 1 h (TLC showed that the reaction was completed). After concentration, the residue was dissolved in dry dichloromethane (2 mL) and EDCI (24 mg, 0.122 mmol), DMAP (catalytic amount), stearic acid (31 mg, 0.126 mmol) were successively added. The mixture was stirred at RT under argon for 3 h. After concentration, the residue was purified by chromatography (1:1 hexane/ethyl acetate) on silica gel affording the desired compound **131** as a white solid (56 mg, 78%); R_f = 0.42 (1:1 toluene-ethyl acetate); $[\alpha]_D -0.43$ (c 0.69, $CHCl_3$); ^1H NMR (600MHz, $CDCl_3$) δ 8.01 (d, 2H, J = 8.4 Hz, ArH), 7.56 (dd, 1H, J = 8.9 Hz, 8.9 Hz, ArH), 7.44 (dd, 2H, J = 7.9 Hz, 7.9 Hz, ArH), 5.87 (ddd, 1H, $J_{5,6a} = J_{5,6b} = 6.8$ Hz, H-5_{sph}), 5.72 (d, 1H, $J_{NH,H-2} = 9.2$ Hz, NH), 5.54 (dd, 1H, $J_{3,4} = 7.25$ Hz, H-3_{sph}), 5.46 (ddd, $J_{4,5} = 15.4$ Hz, H-4_{sph}), 5.34 (dd, $J_{4',5'} = 0.9$ Hz, H-4'), 5.17 (dd, 1H, $J_{3,4} = 9.2$ Hz, H-3), 5.09 (dd, 1H, $J_{2',3'} = 10.3$ Hz, H-2'), 4.95 (dd, 1H, $J_{3',4'} = 3.4$ Hz, H-3'), 4.89 (dd, 1H, $J_{2,3} = 7.8$ Hz, H-2), 4.46-4.52 (m, 1H, H-2_{sph}), 4.46 (d, 1H, $J_{1',2'} = 7.8$ Hz, H-1'), 4.45 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1), 4.36 (dd, 1, $J_{6a,6b}$

= 12.0 Hz, H-6a), 4.13 (dd, 1H, $J_{6a',6b'} = 11.1$ Hz, H-6a'), 4.08 (dd, 1H, H-6b'), 4.00 (dd, 1H, $J_{1a,1b} = 10.1$ Hz, $J_{1,2} = 3.7$ Hz, H-1a_{sph}), 3.97 (dd, 1H, H-6b), 3.86 (dd, 1H, $J_{5',6a'} = J_{5',6b'} = 7.3$ Hz, H-5'), 3.77 (dd, 1H, $J_{4,5} = 9.6$ Hz, H-4), 3.63 (dd, 1H, $J_{1,1} = 10.2$ Hz, $J_{1,2} = 4.6$ Hz, H-1b_{sph}), 3.56 (ddd, 1H, $J_{5,6b} = 5.1$ Hz, $J_{5,6a} = 2.2$ Hz, H-5), 2.16 (s, 3H, COCH₃), 2.07 (s, 3H, COCH₃), 2.05 (s, 3H, COCH₃), 2.02 (s, 6H, COCH₃), 2.00-2.04 (m, 2H, alkane CH₂), 1.96 (s, 6H, COCH₃), 1.56-1.62 (m, 2H, CH₂CH₂C(O)), 1.20-1.40 (m, 52H, alkane CH₂), 0.88 (t, 6H, $J = 6.7$ Hz, 2xCH₃); ¹³C NMR (125 MHz, CDCl₃) δ 172.6, 170.3, 170.2, 170.1, 170.0, 169.6, 169.6, 169.0, 165.2, 137.5, 133.0, 130.3, 129.6, 128.4, 124.6, 101.1, 100.4, 76.0, 74.1, 72.7, 72.6, 71.8, 71.0, 70.7, 69.1, 67.5, 66.6, 61.9, 60.8, 50.7, 36.9, 32.3, 31.9, 29.7, 29.7, 29.6, 29.6, 29.6, 29.5, 29.5, 29.4, 29.3, 29.2, 28.9, 25.7, 22.7, 20.8, 20.7, 20.6, 20.6, 20.5, 14.1; ESI HRMS m/z Calcd for C₆₉H₁₀₉NO₂₁Na: 1310.7384. Found: 1310.7386.



***O*-(β-D-galactopyranosyl)-(1→4)-*O*-(β-D-glucopyranosyl)-(1→1)-(2*S*, 3*R*)-**

2-octadecanamido-(4*E*)-octadecene-1,3-diol (124)

To a solution of compound **131** (30 mg, 0.023 mmol) in methanol (2 mL) was added 2M NaOMe (0.1 mL). The mixture was stirred at RT for 18 h and the reaction was quenched by amberlite IR-120 acidic resin. After filtration and concentration, the residue was purified by silica gel chromatography using methanol in dichloromethane to afford the desired product **124** as a white solid (19 mg, 93%): $[\alpha]_D -12.0$ (c 0.23, pyridine); {lit.^[179]

$[\alpha]_D -5.8$ (c 1.0, pyridine)); ^1H NMR (600 MHz, CD_3OD) δ 7.48 (d, 1H, $J_{\text{NH},\text{H}-2} = 7.7$ Hz, NH), 5.64 (ddd, 1H, $J_{5,6a} = J_{5,6b} = 6.6$ Hz, H-5_{sph}), 5.41 (dd, 1H, $J_{4,5} = 15.3$ Hz, H-4_{sph}), 4.30 (d, 1H, $J_{1',2'} = 7.7$ Hz, H-1'), 4.24 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1), 4.15 (dd, 1H, $J_{1a,1b} = 10.0$ Hz, $J_{1a,2} = 4.2$ Hz, H-1a_{sph}), 4.04 (dd, 1H, $J_{3,4} = 7.7$ Hz, H-3_{sph}), 3.94 (dddd, 1H, $J_{2,3} = 7.7$ Hz, H-2_{sph}), 3.80-3.84 (m, 3H, H-4', H-4, H-6a), 3.79 (dd, 1H, $J_{6a',6b'} = 11.6$ Hz, $J_{6a',5'} = 7.2$ Hz, H-6a'), 3.69 (dd, 1H, $J_{6b',6a'} = 11.6$ Hz, $J_{6b',5'} = 4.4$ Hz, H-6b'), 3.49-3.56 (m, 5H, H-1b_{sph}, H-3, H-2', H-5', H-6b), 3.45 (dd, 1H, $J_{3',2'} = 9.7$ Hz, $J_{3',4'} = 3.3$ Hz, H-3'), 3.35 (ddd, 1H, $J_{5,4} = 9.7$ Hz, $J_{5,6a} = J_{5,6b} = 3.4$ Hz, H-5), 3.28 (m, 1H, H-2), 2.12 (t, 2H, $J = 7.8$ Hz, $\text{CH}_2\text{C}(\text{O})$), 1.95 (m, 2H, alkane CH_2), 1.50-1.60 (m, 2H, alkane CH_2), 1.15-1.70 (m, 50H, alkane CH_2), 0.84 (t, 6H, $J = 6.7$ Hz, alkyl $2\times\text{CH}_3$); ^{13}C NMR (125MHz, CD_3OD) δ 176.0, 135.6, 130.7, 105.1, 104.3, 81.1, 77.0, 76.4, 76.2, 74.9, 74.7, 73.3, 72.6, 70.3, 69.9, 62.8, 62.1, 54.6, 37.8, 33.7, 33.2, 31.0, 30.9, 30.9, 30.9, 30.8, 30.7, 30.6, 30.6, 27.3, 23.9, 15.1; ESI HRMS m/z Calcd for $\text{C}_{48}\text{H}_{91}\text{NO}_{13}\text{Na}$: 912.6382. Found: 912.6375.

Chapter Eight

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